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PRACTICAL CLINICAL LABORATORY DIAGNOSIS

PRACTICAL CLINICAL LABORATORY DIAGNOSIS

A Thoroughly Illustrated Laboratory Guide Including the Interpretation of Laboratory Findings Designed for the Use of Students and Practitioners of Medicine

BY

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ILLUSTRATED WITH 134 BLACK AND WHITE TEXTUAL FIGURES AND 20 PLATES, 9 OF WHICH ARE IN COLORS

THIRD EDITION, COMPLETELY REVISED

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PREFACE TO FIRST EDITION

Several years of teaching Clinical Laboratory Diagnosis, making laboratory examinations and often interpreting the findings, has forcibly impressed us with the need of such a book as this is intended to be.

We take up only those laboratory examinations that are useful in ordinary, every-day practice and do not include tests that are seldom used or that are practical only for special laboratory workers.

Our plan throughout the book is to give only one method of making a test or examination. We select what we consider the best, simplest, most practical method of making each test and do not sacrifice space or clearness by giving several tests or methods for obtaining the same information. All apparatus employed are specified, usually illustrated, and when thought to be advantageous, a source of supply considered reliable is given. Reagents are all specified, the formulas given, and an economical and practical source of supply is stated when thought advantageous.

Some of the technic is original. Many of the "short cuts" and simplified steps are our own personal adaptation of the work of others. We wish to give full credit here and to state that all we know of laboratory diagnosis or any other subject has been learned either directly or indirectly from the work of others. All the illustrations and colored plates are original. They are accurately drawn and colored, frequently with the aid of the camera lucida. It often occurs that a *thing* can be illustrated better by pictures than by written description. Written descriptions are sometimes too technical to be easily understood or too long for convenience. A single picture often shows as much as can be described on a page or more of print. The picture can be read at a glance, while the page of print requires minutes to read.

In an appendix we give a list of the necessary material and apparatus required for the laboratory diagnoses described in the text. To do all ordinary laboratory tests one requires only a very limited amount of material and apparatus, and a knowledge of how to use

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them. We are not advertising for anybody, but wherever deemed advisable we have not hesitated to recommend apparatus or material of a particular make or from a source considered reliable.

In the text we give, as fully as space will permit, the interpretation and relative value of the different findings. It would, of course, be impossible to discuss thoroughly in a work of this kind every possible indication of all the tests.

Though the book is intended primarily for students and those doing only the ordinary every-day clinical laboratory work, it is believed that laboratory specialists may also find in it some practical suggestions of value to them.

We wish to express our appreciation of suggestions made by many friends and associates, and the opportunities that our positions in the Tulane College of Medicine have furnished which have permitted the experience and observations on which this book is largely based.

The Authors.

PREFACE TO THIRD EDITION

The enthusiasm with which the first two editions were received and the urgent request of so many for another edition, together with the need which we have felt so keenly for this book for the use of our own past, present and future students, led us to undertake this third edition. The former publishers have gone out of business, but we are fortunate to have Williams and Wilkins, whose enviable reputation is well known, take over the publication.

The book has been entirely rewritten and reillustrated from cover to cover, only such parts of former editions being retained as we have found no occasion to change. Where improved methods have been developed and proved, we have not hesitated to use them.

While we have adhered to the original idea of limiting this work to only the clinical laboratory procedures that are practical and are required in efficient routine daily practice of medicine, we have included a number of subjects and tests not included in former editions.

We have adhered strictly to the same general idea as in the first edition, of selecting what we consider the best, simplest, and most practical method of making each test and not sacrificing space or causing confusion by giving several tests or methods for obtaining the same information.

The kindness is appreciated of those who have pointed out errors in former editions and made valuable suggestions. The fact that the book has passed through two large editions amounting to several thousand copies, which have long since been exhausted, practically without any sales promotion, leads us to hope that this revised, rewritten and greatly improved edition will serve a useful purpose.

The Authors.

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CHAPTER I

Use and Care of the Microscope

The microscope is used in a large part of the examinations made in the clinical laboratory. Therefore it is important to have a good microscope and to know how to use and care for it. The best microscope will be of little service to one who does not know how to use a microscope properly.

Selection of a microscope. There are several manufacturers of microscopes, each making several different models. The following is a good selection to meet the needs of a physician in medical laboratory work:

Bausch and Lomb model "FFSA 8" (Fig. 1). Objectives of 16 mm. ("low dry"); 4 mm., N.A. 0.65 ("high dry"); and ~~1.0~~ 1.9 mm. ("oil immersion") with triple nosepiece. Oculars, 10× and 5×. Abbé condenser, with lower iris diaphragm only. Mechanical stage to fit, built on but detachable, without vernier. Bausch and Lomb Optical Company, Rochester, New York.

Corresponding microscopes, very similar in every partcular to the one just described, are manufactured by Spencer Lens Company, model 44 M-H (Fig. 2) and also by E. Leitz, model LS 10 (Fig. 3), either of which is entirely satisfactory for our purpose.

The binocular microscope permitting the simultaneous use of both eyes has many advantages, such as more detail to be seen with both eyes, stereoscopic effect, relief of eye-strain and less concentration required on the part of the observer. We strongly recommend it in preference to the monocular microscope to all who can conveniently afford the difference in price, about $70. The Bausch and Lomb model "FFSEA 8" (Fig. 4) or the Spencer model 4 M-H (Fig. 5) or the Leitz model LBMS-10 (Fig. 6) are suitable for our purpose.

If dark-field work is to be done (and it should be done, if diagnosis of suspected syphilitic lesions is to be made), a dark-field condenser with attached substage light (Fig. 120) to fit in the substage of the particular microscope, and a suitable funnel stop (Fig. 122) for the

oil immersion lens, should be purchased at the same time. If ordered later, give the manufacturers the factory number and exact description of your microscope.

Cleaning the lenses. The exposed surfaces of oculars, objectives,

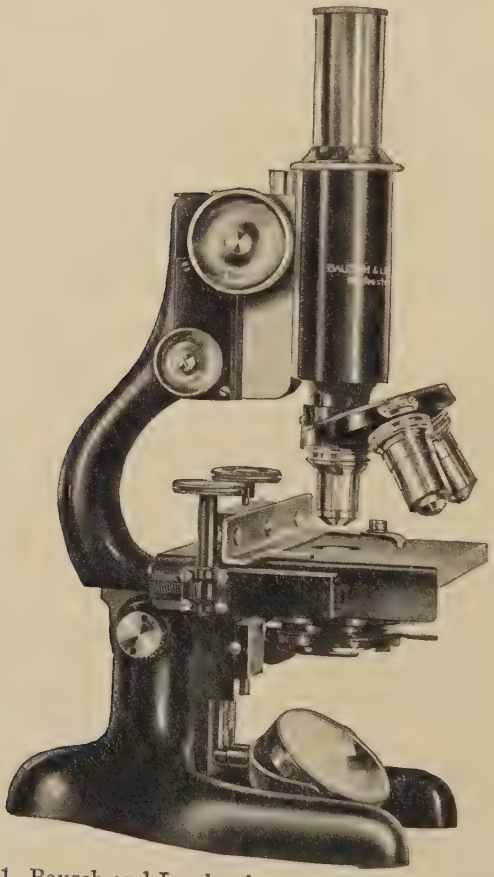


Fig. 1. Bausch and Lomb microscope, model "FFSA 8"

condensers and mirrors are likely to have dust particles collect upon them at any time. It is best to wipe them off with a soft linen cloth (handkerchief) just before use each time. Keep the ocular and objectives attached to the microscope all the time, and there is

little danger of dust getting on the back lenses of the objectives and lower lens of the ocular. They require cleaning only occasionally.

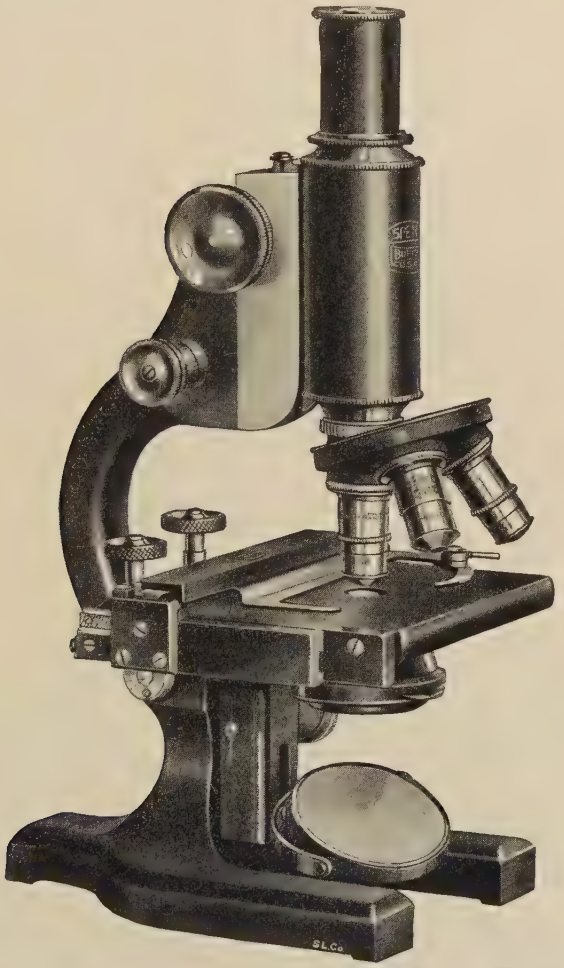


FIG. 2. Spencer microscope, model 44 M-H

Sometimes the *inner* surfaces of the lenses in the ocular require cleaning due to a film that forms on them. This you can do. Objectives should be sent to the makers every three to five years or

oftener, if necessary, to be cleaned. Don't try to clean them on the inside. You should clean the *back lens* of the objectives occasionally by wiping with a suitable piece of cloth wrapped on a small wooden stick or applicator, or with the corner of the cloth rolled into proper shape.

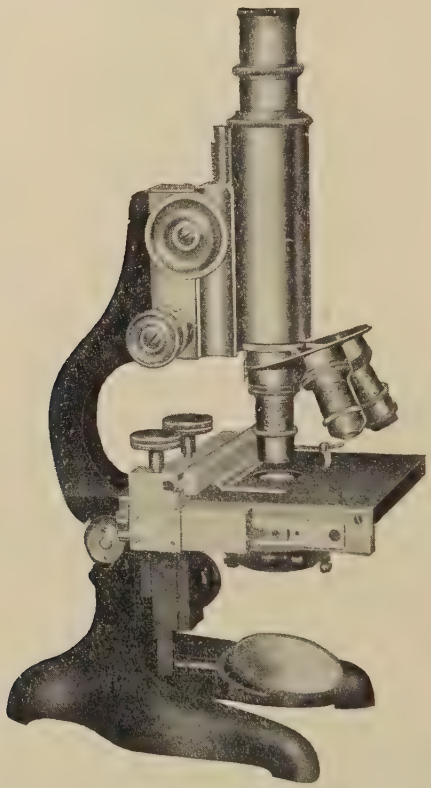


Fig. 3. Leitz microscope, model LS-10

Care of the microscope stand. It is better to keep the microscope assembled and on the table ready for use. A bell jar is a good cover to protect it from dust. Wipe it occasionally with a slightly oily cloth. Wipe off any gummy oil on the bearings and oil them *a little* with soft tallow or white vaseline. If gummed too badly, remove by wiping the bearings only with cloth moistened with xylol.

You can put a little oil in the bearings of the coarse adjustment. If anything gets wrong with the fine adjustment, send the microscope to the maker for repairs. Don't try to repair it yourself.

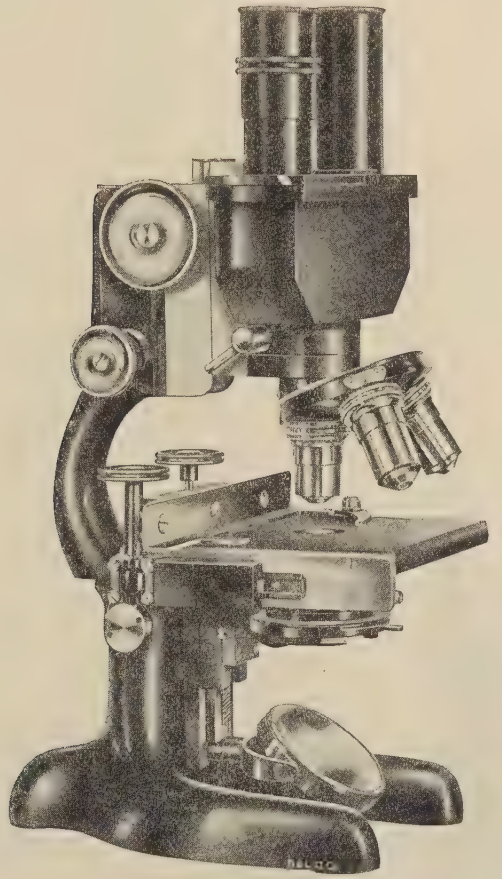


FIG. 4. Bausch and Lomb mon-objective binocular microscope, model "FFSEA 8."

Clean and oil the mechanical stage in the same way that you do the stand. Be sure to put the mechanical stage on so that it does not bind and drag on the stage of the microscope.

Correct position at the microscope. Do not tilt your microscope

stand. Sit up straight, close to the table, having the base of the microscope near the edge of the table (Fig. 7). Keep both eyes open.

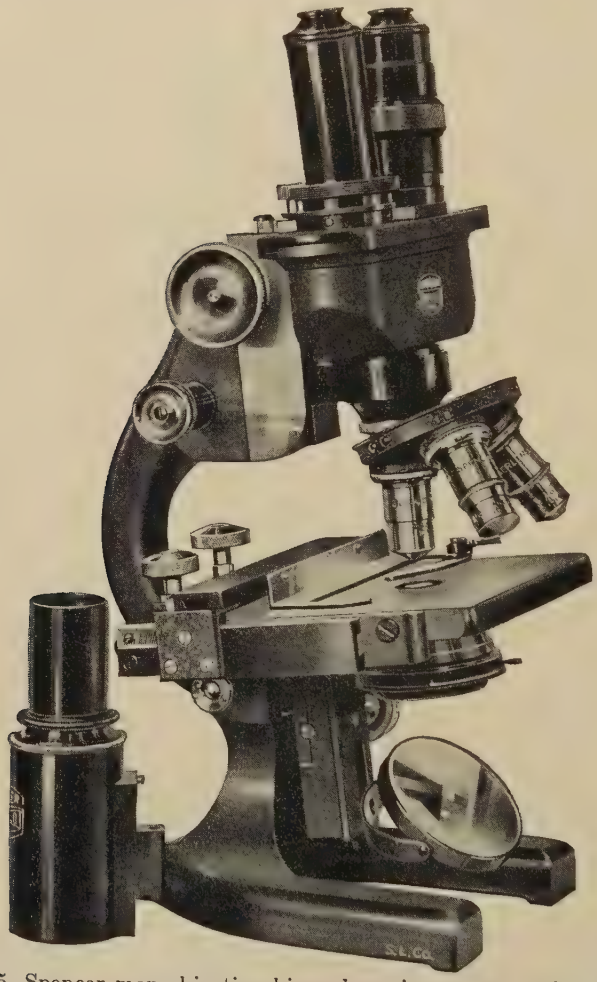


FIG. 5. Spencer mon-objective binocular microscope, model 4 M-H

To learn to do this, in using monocular microscopes it is best to first turn the head to one side considerably (Fig. 8) and to gradually bring it back straight. A few hours' practice is usually sufficient to

learn to keep both eyes open while looking in the microscope with one. Learn to look in the microscope with either eye.

Source of light. A window on the north side of the room is best. Where electric lights are available they give the most uniform light and have the further advantage of being usable any time of day or

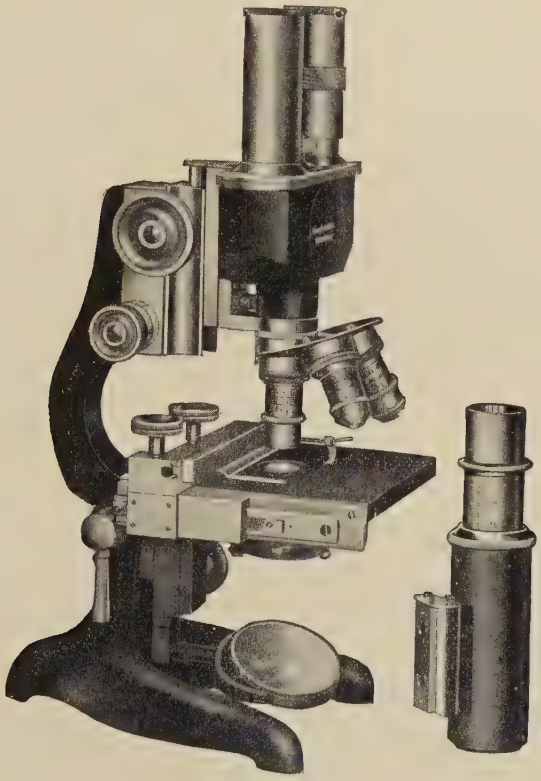


Fig. 6. Leitz mon-objective, binocular microscope, model LBMS-10

night. A mazda lamp with round frosted globe is best. If some such lamp-holder as shown in Fig. 9 is used so the light can be placed *near* the microscope, a 25-watt mazda lamp will give sufficient light, otherwise use a 50-watt lamp.

Adjustment of the mirror. The mirror has one concave and one plane surface. When used without the Abbé condenser Fig. 10 (which

FIG. 7. Proper position at the microscope. Both eyes open. Body erect

FIG. 8. Learning to look in the microscope with both eyes open. The head is turned to one side.

is seldom done in our work), the concave surface gives the strongest light by concentrating the rays, while the plane surface gives the least light. These conditions are reversed when the Abbé condenser

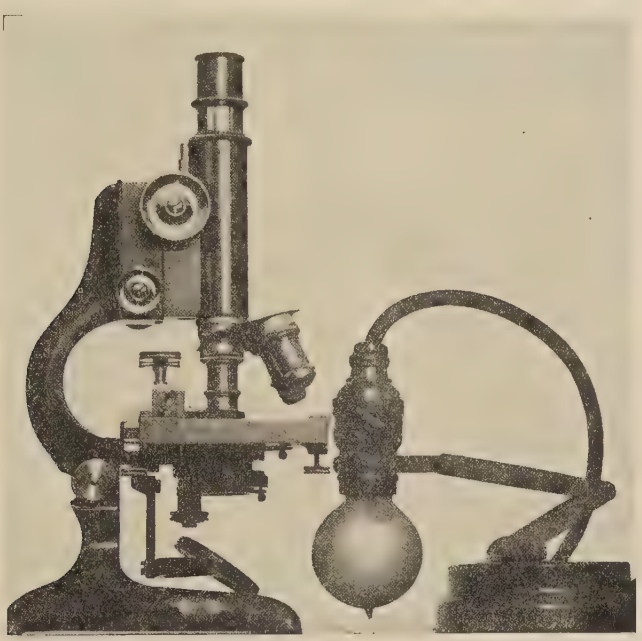


FIG. 9. A good light (Mazda lamp, frosted globe, 25 watt) and adjustable light holder in proper position in front of the microscope.

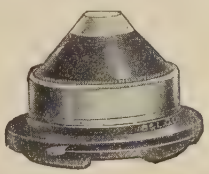


FIG. 10. Abbé condenser removed from the substage. This can be easily slipped out of its carrier. The iris diaphragm is opened or closed by the lever shown at the left lower side of the picture.

is being used (Fig. 11). The plane surface should be used, therefore, practically all the time. Keep the microscope directly in front of you, adjusting the mirror rather than the microscope stand, to obtain the best light. The mirror must be adjusted so that the best light

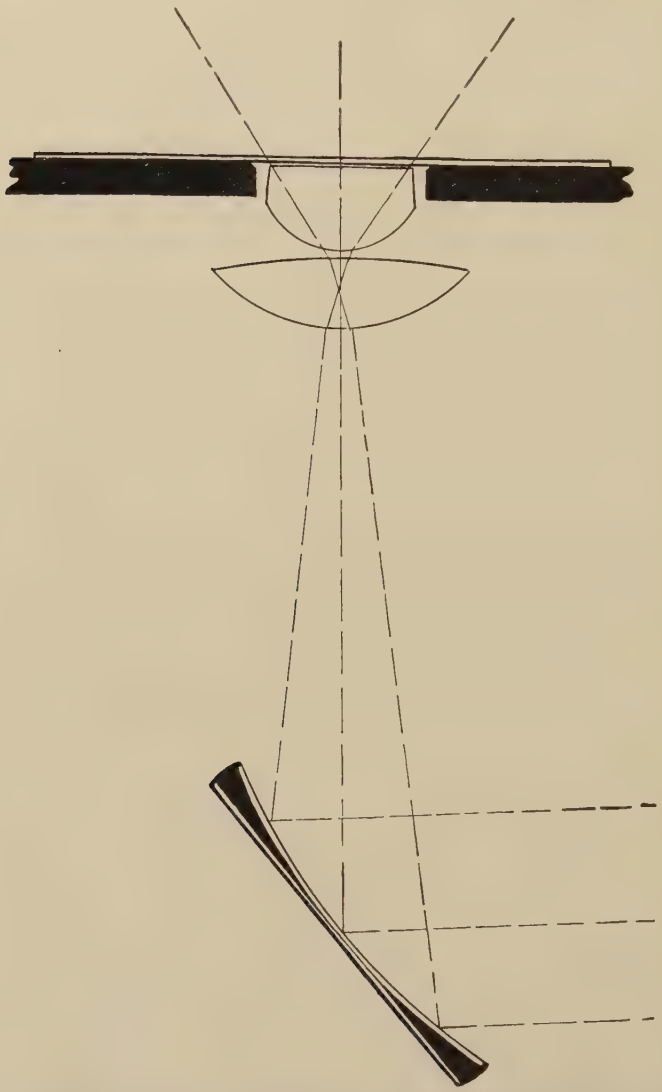


FIG. 11. Path of light rays reflected from the concave mirror through the Abbé condenser. Note that the light comes to a focus in the condenser and not above it, as is desirable in most cases. The concave mirror should seldom if ever be used except when the Abbé condenser is thrown out and is not in use.

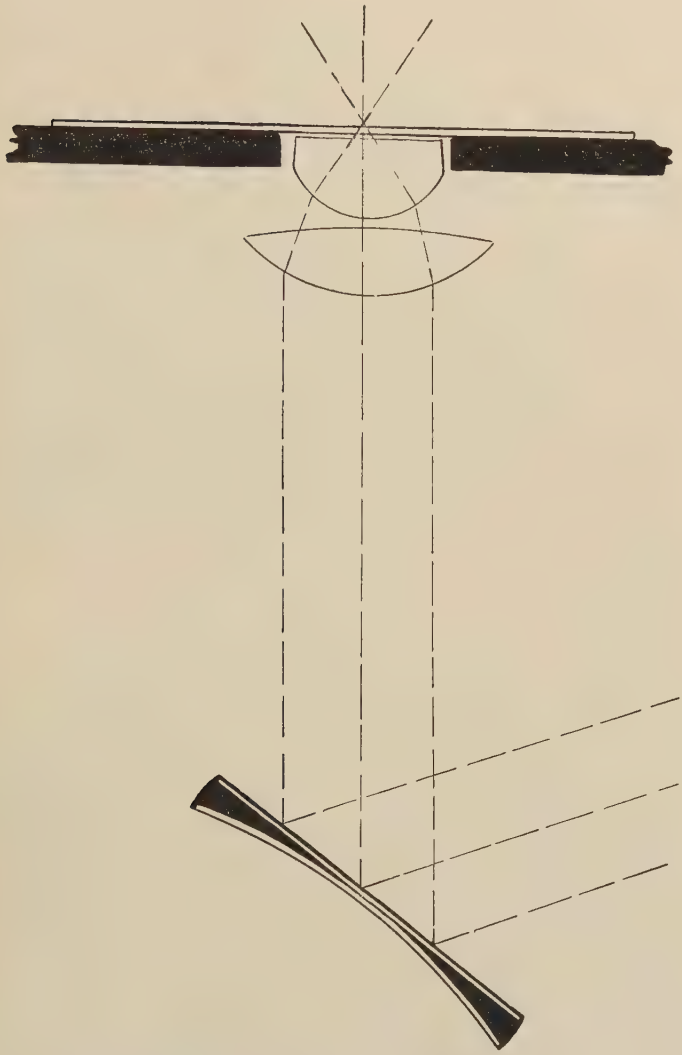


FIG. 12. Path of light rays reflected from the flat mirror and focussed upon the object by the Abbé condenser when in proper position.

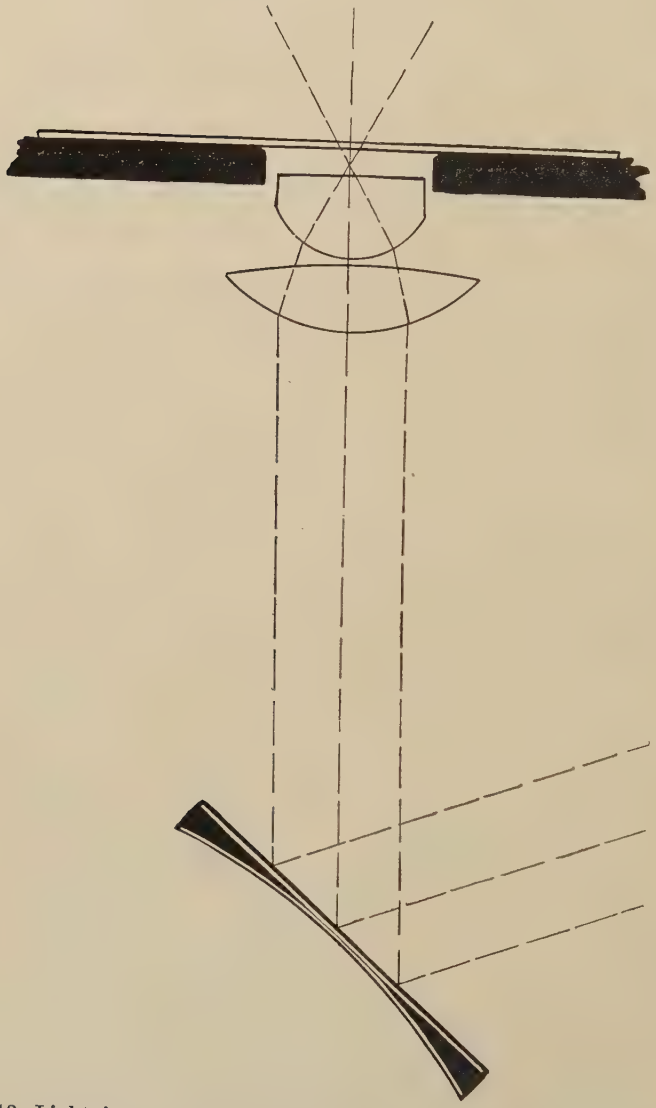
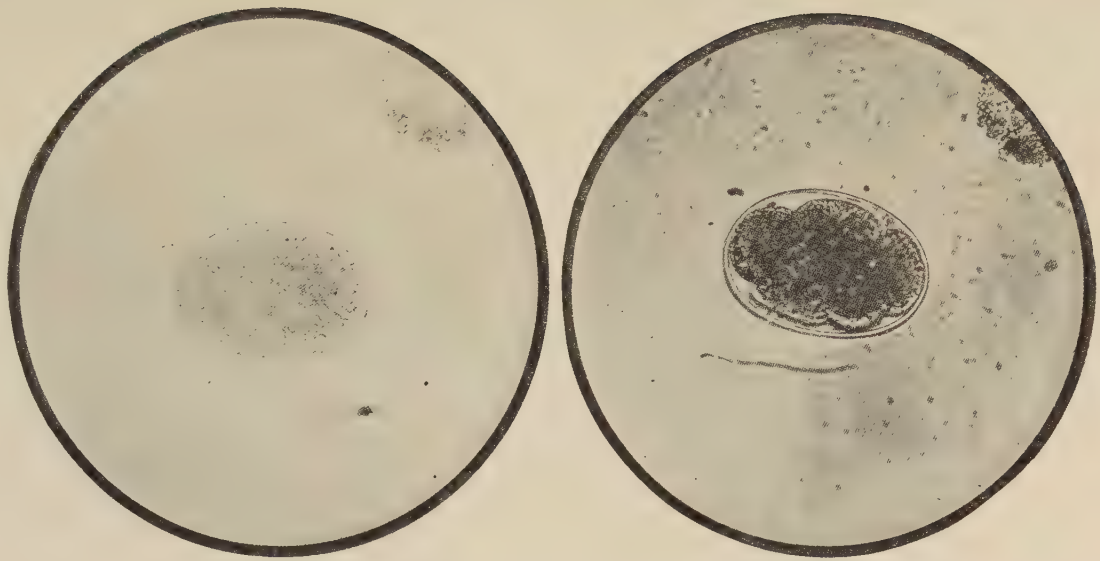


FIG. 13. Light improperly focussed below the object, resulting from the Abbé condenser being too low. It often happens that the condenser is slipped down in the sleeve which carries it and not noticed and the microscope is used at this great disadvantage for some time. Watch out for this.



A B

FIG. 14. Photomicrographs of the same field. A. Too much light. Note that the hookworm egg is poorly shown and other objects cannot be seen at all. B. Proper amount of light obtained by reducing the size of the opening in the fris diaphragm of the condenser.

is directed through the object. This is determined by tilting the mirror from side to side while looking in the microscope. Note the point at which the brightest light is obtained. The mirror must be readjusted every time the microscope is moved on the table.

The Abbé condenser. The Abbé condenser (Fig. 10) serves the purpose of concentrating the light rays and directing them at a different angle through the object. It is carried in a slip sleeve in the substage. It frequently gets pushed too low in this sleeve and this is a great source of poor illumination. Refer to Figs. 12 and 13 and note that proper illumination is obtained by having the surface of the condenser approximately as high as the surface of the stage of the microscope. Examine your Abbé condenser and learn how it can be moved up and down, and look out for its getting out of place. There is an iris diaphragm under the condenser with which the amount of light must be regulated. It is strange how long it takes students to learn to appreciate the necessity of regulating the light for different objects. In practice, the light should be adjusted and regulated for practically every slide examined (Fig. 14), and frequently many times in examining a single specimen. *Remember this.* Generally speaking, the higher the magnification, the more light required. With low power lenses, it is necessary to reduce the light. Also the more color an object has, the more light is required. Hyaline and unstained objects require less light.

Focusing. Place the part of the object to be examined approximately in the center of the field, which can be guessed by its position over the Abbé condenser. With the coarse adjustment, bring the tip of the objective near the object; then look in the microscope and rack upward until the image appears. Then focus with the fine adjustment. Manipulate the fine adjustment with the left hand and the mechanical stage with the right (Fig. 7).

Use of oil immersion lenses. Only the best cedar oil "for immersion" should be used. It gets gummy and colored upon long exposure to light and air. Don't use such oil. Don't let dust get into it. Put a small drop of oil on the specimen over the place selected to examine. Run the tip of the oil immersion lens down until it touches the oil. Now focus as with other objectives. After use, the oil must be cleaned off. To do this wipe the lens carefully with a soft linen cloth. A soft handkerchief is satisfactory for this purpose. Never let oil dry on the lens. If this happens, remove it with a cloth moistened with xylol.

CHAPTER II

Blood

GENERAL CONSIDERATIONS

Obtaining blood for all microscopic examinations. Some kind of a "blood sticker" is required. A good and convenient one may be made of a straight Hagedorn needle No. 6. The point is usually too long and narrow. Grind it to a proper short angle. Stick the eye end of the needle into a cork stopper and insert it into a 2-drachm homeo vial to carry the needle in when not in use (Fig. 15). Do not put any antiseptic solution in this vial. Needles should be kept dry. A still better "blood sticker" illustrated by Fig. 16 consists

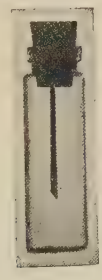


Fig. 15. A good "blood-sticker." A properly shaped and sharpened Hagedorn needle No. 6, in 2 dram homeo-vial.

of a rustless steel blade and metal handle and holder. This costs more, but we recommend it as first choice.

The side of the first phalanx of one of the fingers is a convenient place to draw blood from. This is especially true when taking blood from yourself. One should learn to take blood and make the spreads with his own blood. It is sometimes more convenient to draw blood from the ear-lobe of patients. The principles of the technic are the same.

Wipe the skin dry at the site it is intended to stick. When obtaining blood from a patient, it is good practice to wipe the needle and the site to be punctured with a pledget of cotton or gauze

moistened with alcohol. Squeeze the place to be stuck between the thumb and finger and make a quick stick with the needle while squeezing. The pressure lessens pain sense. Most workers can govern the depth and accuracy of the stick better if they steady the hands by letting them rest upon a table or other surface when sticking a finger (Fig. 17), or against the side of the face and neck of the patient when sticking the ear (Fig. 18). The puncture should not

Fig. 16. Ideal "blood sticker." Stainless steel needle. (See appendix for source of supply.)

Fig. 17. Squeezing finger when sticking to steady it and to lessen pain sense.

be deep enough to cause blood to flow without proper manipulation of the surrounding tissue. It should be made so quickly and well that the patient hardly knows when it is done.

To squeeze out the blood, use both hands. Try to entirely surround the end of the finger (Figs. 19 and 20) or ear-lobe (Fig. 21) with your fingers so that the blood cannot escape backward. Your fingers should not be nearer than from one-fourth to one-half inch

of the "stick" (Figs. 22 and 23). If you hold and press too close to the "stick," little blood will be obtained. Hard squeezing dilutes the blood with tissue "juice" and must be avoided. Squeeze out the proper amount with which to make one spread. About one-fourth drop is the proper amount. Stop the pressure and no more blood will flow if the "stick" is of the proper size and depth. If the skin is dry, the blood will stand up properly as a small round mass, but

FIG. 18

FIG. 19

FIG. 18. Squeezing the ear-lobe and steadying the hand against the face of the patient when making the stick.

FIG. 19. First step, after making the stick, in obtaining blood from yourself. The blood is prevented from running back by constriction made around the finger by squeezing it in this manner.

if it is not, the blood will spread out on the skin. The proper amount of blood is taken up on a clean slide by touching the slide to it. Do not let the slide touch the bleeding finger. It is best, for most operators at least, to steady the hand which holds the slide against the table (Fig. 24), or the other hand (Fig. 25), or the face (Fig. 26) of the patient when taking blood from the finger or ear, before touching the slide to the drop of blood. The blood is now spread out upon the slide and then another proper amount of blood

is squeezed out and spread upon another slide in the same way. It is best to make at least two good preparations, and preferably three, in every case. If a proper "stick" is properly manipulated, twenty-five to fifty preparations can be made from it if desired.

FIG. 20. First step in squeezing out blood, constricting the patient's finger after the puncture has been made.

FIG. 21. Squeezing out blood from the ear lobe. Note that the pressure is not made close to the puncture.

Making blood spread for differential leukocyte count, examination for malaria plasmodia, looking for abnormal cells, making typhoid agglutination test, etc. The advantages of well made blood spreads are sufficient to justify the effort necessary to learn to make them.

FIG. 22. Second step in squeezing out blood from one's own finger. Note that the pressure is not made near the site of the puncture. The pressing fingers of the right hand are held nearly parallel to the bleeding finger, and pressure is made in this manner while the blood is prevented from returning by the constriction made by the fingers of the left hand.

FIG. 23. Squeezing blood from the patient's finger. Note that pressure is not made close to the puncture.

There are comparatively few who do make good ones. A general purpose blood spread should have both thick and thin portions, and there should be no dragging of the larger cells. The blood should be spread upon the middle third, or a little more, of the slide and should not reach to either end (Fig. 27).

Have the slides clean and free from chemicals. When not soiled with oily or gummy material and stains, they may be washed in water and wiped dry with a towel or cloth. *Hold by the edges* when

FIG. 24. Taking up blood from patient's finger. The slide is first steadied against your own forefinger or thumb which is holding the patient's finger.

FIG. 25. Taking up blood from your own finger. The edge of the slide is first steadied against your forefinger.

wiping. Oily or stained slides may be cleaned by boiling them in water to which has been added a little washing powder or soap, and subsequently rinsing well and wiping dry.

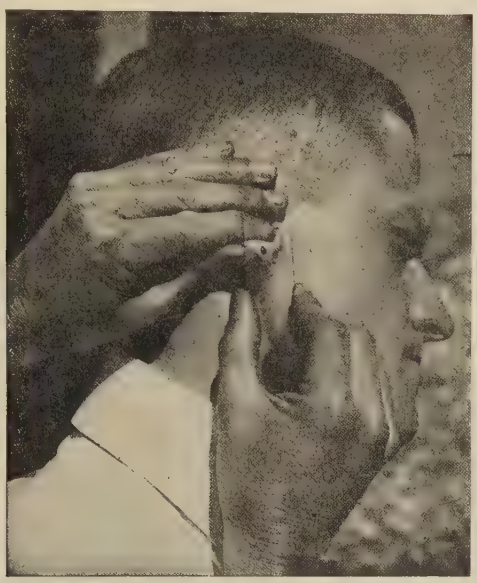


FIG. 26. Taking up blood on slide from ear-lobe of patient. This picture erroneously shows the blood about to be taken up on the wrong end of the slide. It should be taken on the lower end.

FIG. 27. A good general purpose blood spread labelled by writing on the blood smear with an ordinary lead pencil.

Take up the blood on the slide at about three-fourths of an inch from one end. Hold the hand in the position shown in Fig. 28, ready to receive the slide. Place the end of the slide farthest from the blood drop, between the thumb and finger (Fig. 29), and bring up the little finger to support the other end, or lay the slide down on the table before you. Spread the blood with another slide held in the other hand. Place the end of the spreader slide just in front of the drop of blood and bring it back to it (Fig. 30). Hold it

FIG. 28

FIG. 29

FIG. 28. Proper pose of hand and fingers to receive the slide with blood on it.
FIG. 29. Slide with drop of blood on it held in proper position for spreading.

at an angle of about thirty degrees (Fig. 31). The blood spreads toward both edges and should all be behind the sharp edge of the end of the spreader slide. As soon as the blood has spread entirely across the end of the spreader slide, with a rather quick movement, push (Fig. 32) the spreader slide toward the end of the lower slide (Fig. 33), spreading out the blood in a thinner or thicker film, according to whether the movement is slow or rapid, respectively. It is desirable to leave all of the blood on the middle third of the slide. Note that the last part of the spread (Fig. 27) is considerably

thinner than the first part. Such a spread offers fields of any desired thickness. One made with too slow a motion, resulting in a very thin preparation, will always show dragging of the leukocytes, which alters the differential count. Allow the blood to dry. The preparation may be labeled by writing on the blood with a pencil after it has dried. Such slides will keep for several days unstained,

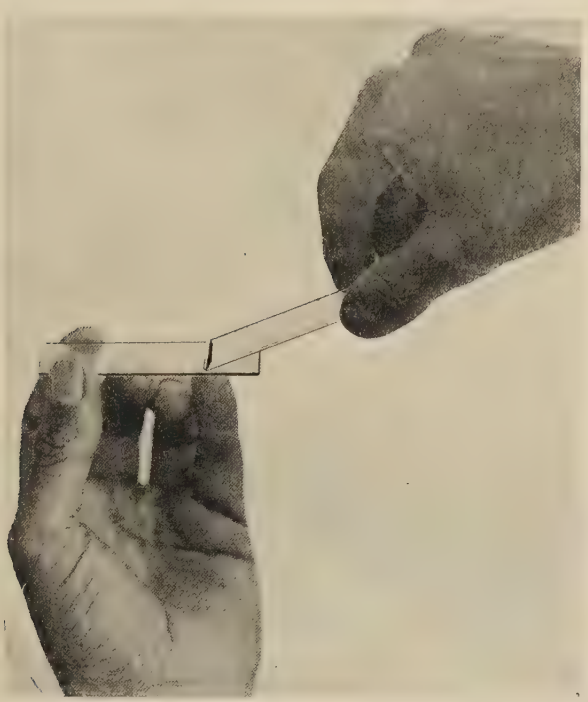


FIG. 30. Slides held in proper position preparatory to spreading blood.

if not convenient to stain them earlier. However, you will generally get the best stains on fresh specimens.

Staining blood slides with Wright's stain. There are several good methods of staining blood. We prefer Wright's stain. It is a good one. Wright's stain is a solution of a mixture of eosinate of methylene blue and eosinate of methylene azur, prepared in a certain way

described by J. H. Wright (*Journal A. M. A.*, 1910, Vol. LV. . 1979) dissolved in pure methyl alcohol (Formula 1).

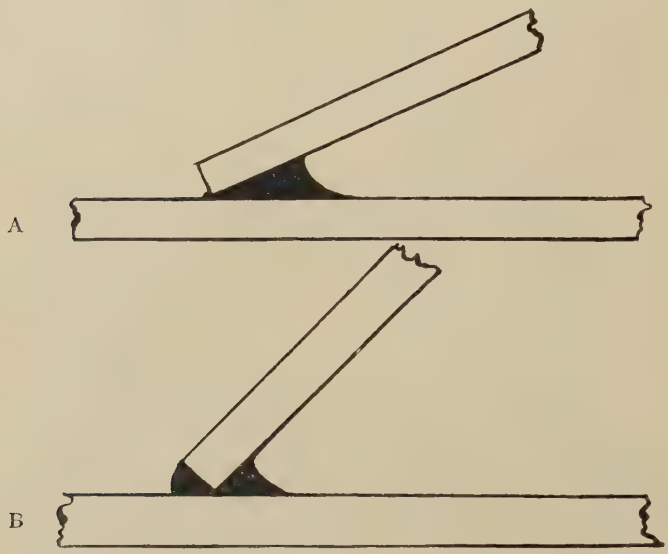


Fig. 31. A. Indicating how blood collects in the acute angle formed by the spreader slide when the latter is held at proper angle. B. Indicating how blood collects in front of the end of the spreader slide when the latter is held at too great an angle. This should be avoided.

Fig. 32. Angle at which slides should be held and the direction in which the spreader slide should be moved in spreading the blood.

Technic of staining. 1. Place slide on staining bar over waste jar, as shown in Fig. 34.

2. Pour on Wright's stain to cover entire slide and allow to remain for one minute. The staining bar should be level.

3. Dilute stain with about two or three times as much *distilled* water and allow to stain from 5 to 10 minutes.

4. Rinse off with distilled water.

FIG. 33. The blood has been spread by pushing (not pulling) the spreader slide quickly. All of the blood is left spread out smoothly on the middle third of the slide or a slightly larger area.

5. Shake off excess water. Wipe off water and precipitated stain on back and front of slide, taking care not to disturb the blood specimen. Stand on end to dry (or fan in air to hasten drying). *Do not blot.*

The specimen can be examined while wet, under the low power lens, for correctness of stain, which is indicated by the nuclei having

a deep, rich purple color and the cytoplasm of the small mononuclears a deep blue.

The amount of stain poured on the slide should be the minimum that will cover the film well and not evaporate sufficiently to throw down a precipitate on the slide in the one minute it is allowed to act.

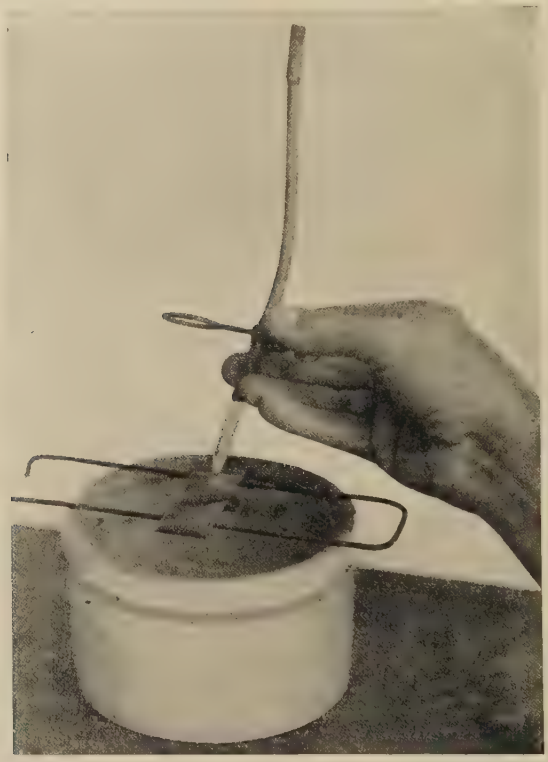


Fig. 34. Diluting Wright's stain on slide; slide resting upon the slide-rest over a waste jar; water from water bottle. Note how the pinch-cock and tube are held. This is a good way.

Add the water from the water bottle (Fig. 34), the tube from which should hang over the staining bar and jar, and should be just long enough for the tip to clear the slide about an inch. By noting the amount of staining fluid on the slide one can guess when he has added two or three times as much water. It is often necessary to vary

the quantity of water added somewhat with different samples of staining solution. It is also sometimes necessary to vary the length of time the diluted stain is allowed to remain on the slide, with different samples. A good plan when beginning the use of a stain with which you are not familiar is to stain several slides different lengths of time, say from one to ten minutes, and see which is the best. The water used to dilute and wash with must be either distilled or at least of high purity. Rain water is usually good.

CHAPTER III

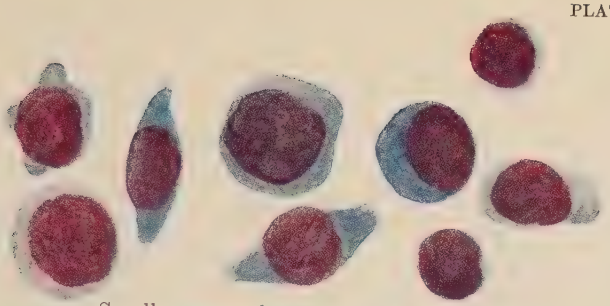
Blood—*continued*

DIFFERENTIAL LEUKOCYTE COUNTS

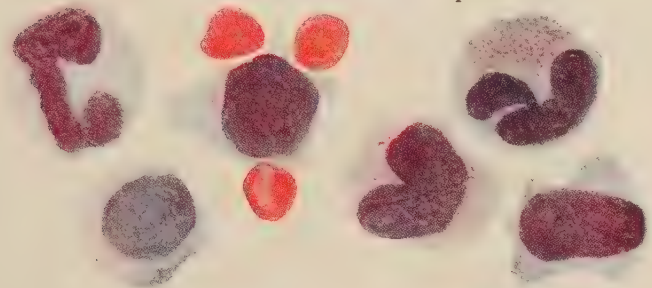
Description of the leukocytes found in normal blood. There are at least five different kinds of leukocytes present in normal blood. They are differentiated from each other by the size, shape and staining reaction of the nuclei, cytoplasm and granules present in the cytoplasm of some of them. The classification which we believe most practical, in the light of our present information, is as follows:

A. Small mononuclear leukocytes (lymphocytes). These cells (Plate I) have a single round or oval nucleus which is frequently indented on one side. They have a relatively small amount of cytoplasm. Some have only a narrow band surrounding the nucleus, while others have very much more. They vary in size from about the diameter of an erythrocyte to nearly the size of a neutrophile. The nucleus is stained a beautiful purple in well-stained specimens and the cytoplasm varying shades of deep blue. The nucleus and cytoplasm are sharply differentiated. In the cytoplasm of a good many of the lymphocytes, there are from one to several reddish stained granules. These cells constitute about 20 to 30 per cent of all the leukocytes in the blood of normal adults.

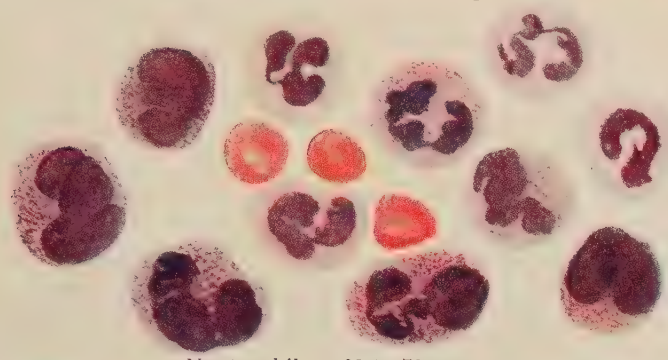
B. Large mononuclear leukocytes ("monocytes," "clasmatocytes," "endothelial leukocytes," "transition cells"). These cells (Plate I) vary in size from that of neutrophiles to considerably larger. Both the nucleus and cytoplasm appear to be less dense than those of the small mononuclear leukocytes, and frequently the cytoplasm is not so clearly differentiated from the nucleus. The proportion of the cytoplasm to nuclear material is much greater than in the small mononuclear leukocytes. The nucleus stains purple and the cytoplasm varying shades of light blue. In deeply stained specimens the cytoplasm of the older cells approaches the lavender of the neutrophile, making the differentiation depend largely upon size and amount of cytoplasm. The nucleus varies in shape from round



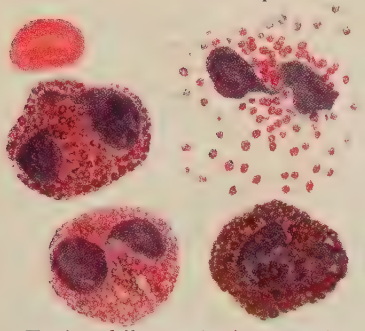
Small mononuclears—20 to 30 per cent



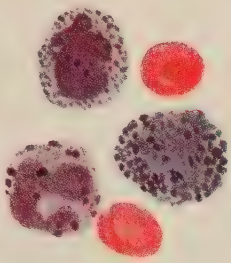
Large mononuclears—1 to 8 per cent



Neutrophiles—60 to 70 per cent



Eosinophiles—1 to 4 per cent



Basophiles—1 per cent

LEUKOCYTES

or oval to slightly lobulated. It is often placed more or less concentrically. Some of the large mononuclear cells contain no granules, but most of them do show from a few to many purple-stained granules in their cytoplasm. These vary in size between that of the granules in neutrophiles and those in eosinophiles. The large mononuclear leukocytes constitute about 1 to 8 per cent of all the leukocytes in the blood of normal adults.

C. Polymorphonuclear neutrophilic leukocytes. These cells (Plate I) are from two to nearly three times the diameter of erythrocytes. The nucleus stains purple and is polymorphous, each one being somewhat different in shape from every other one. The outline of the nucleus is very irregular, and most of them are more or less lobulated. When studied carefully under high magnification, the nucleus appears to consist of a string or ribbon of nuclear material wadded up in the cytoplasm of the cell. Sometimes the bands connecting the lobes or masses are so very thin that they can be made out only with difficulty. In other (younger) cells the nucleus is horseshoe-shaped.

The cytoplasm does not stain, but it contains many fine granules which do stain purple, some taking more of the red than others. In speaking of them, it is common to call them neutrophiles, dropping the other cumbersome part of the name. The polymorphonuclear neutrophilic leukocytes constitute about 60 to 70 per cent of all the leukocytes in the blood of normal adults.

D. Polymorphonuclear eosinophilic leukocytes. These (Plate I) average very slightly larger than the neutrophiles. Their nuclei resemble very much the nuclei of the neutrophiles, but there is possibly greater tendency for the nucleus to be divided into distinct lobes (2 or 3). The nuclei are often horseshoe-shaped. The cytoplasm does not stain, but there are many large granules embedded in it, which take the eosin in a mixture containing eosin, and the cells are therefore called eosinophilic or eosinophiles. They constitute about 1 to 4 per cent of all the leukocytes in the blood of normal adults.

E. Polymorphonuclear basophilic leukocytes. These cells (Plate I) are slightly smaller than the neutrophiles. The nucleus stains faintly and the lobes are less distinct. It more than half fills the cell. The cytoplasm does not stain. There are a variable number

of large coarse granules which seem to project from the surface. They take the basic stains such as methylene blue and on account of this affinity for basic dyes, the cells are called basophilic, or basophiles. The latter term is generally used. In specimens well stained with Wright's stain these granules take a combination of the dark blue methylene blue and the purple. The basophiles constitute less than 1 per cent of all the leukocytes in the blood of normal adults.

Method of making a differential leukocyte count. A differential leukocyte count is a determination of the relative proportion or percentage of the different kinds of leukocytes present. The ordinary

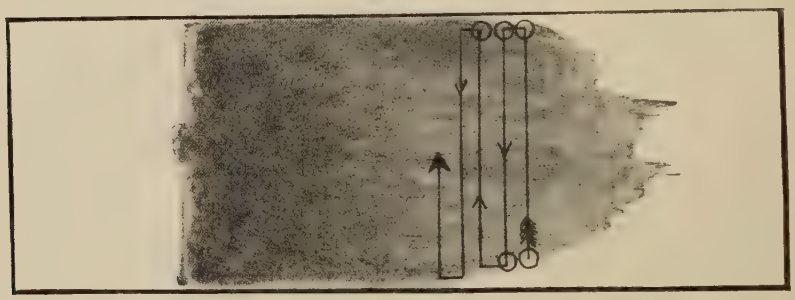


FIG. 35. Proper manner of crossing and re-crossing blood films in making differential leukocyte counts.

blood spread described above (Fig. 27), stained with Wright's stain, is suitable for this purpose. Differential counts are made with the oil immersion lens. A mechanical stage is an advantage. Beginners and those not thoroughly familiar with the different cells should make their counts on a comparatively thin part of the spread where it is not more than one cell thick. After more experience has been gained, more rapid counting can be done on the thicker part of the spread. A good spread has areas varying from too thick to count well, at the thick end, to unnecessarily thin, even for the beginner, at the other end. It frequently takes a long time for beginners to learn to locate proper ground to study. They do not realize the extent of the preparation under such high magnification. The student should learn early to select the proper part of the prepara-

tion. The great difference can be best appreciated by looking over the spread carefully with the low dry lens.

Whenever favorable ground is located, move toward one edge of the spread until the edge is reached (Fig. 35), keeping count of all leukocytes observed. Then move toward one end or the other at least the width of the microscope field, and then across the spread to the opposite edge. In this way cross the spread back and forth, always on new ground, until the desired number of cells have been counted. No cell should be counted twice. All cells passed over must be counted. If they cannot be classified, put them down as "unclassified" cells. At first a good many are found that cannot be classified, but with experience the student is soon able to classify practically all the cells found in normal blood.

In counting, a tally sheet should be provided on which to mark down the number of cells counted. It is not necessary to write out the names of the different cells, as these are familiar terms which can as well be indicated by proper abbreviations. S, may be used for small mononuclear leukocytes; L, for large mononuclear leukocytes, and N, E and B, for polymorphonuclear neutrophilic, eosinophilic, and basophilic leukocytes, respectively. Likewise, U may stand for unclassified leukocytes.

The percentage of each kind of cell can be calculated after any number has been counted, but it is much better to count exactly one hundred cells, in which case the number of each kind of cell counted is also the percentage, and no other calulation is necessary. After a little experience one learns to stop and add up when nearly one hundred cells have been counted.

Instead of stopping to put down each cell, one soon learns to carry the small mononuclears and neutrophiles "in his head" until nearly one hundred have been counted, only stopping to put down the other cells which are few in number. When the number of cells reaches nearly one hundred they are all put down. The following is an example of such a tally sheet:

S	23
L	5
N	66
E	2
B	1
U	

Upon adding up it is seen that three cells are required to make one hundred. These are found and added and the tally sheet completed. It would then look as follows:

S	23	23
L	5	5
N	66 + 2	68
E	2 + 1	3
B	1	1
U		
	97	100

FIG. 36. Looking in the microscope with the left eye in making differential leukocyte counts. The writing down of the number of cells counted can be plainly seen with the right eye without moving the head and taking the left eye away from the microscope.

Another important short step in making differential leukocyte counts is to look in the microscope (Fig. 36) with one (the left) eye

and look at the tallying with the other (the right) eye, without moving away from the microscope. This can be learned with a little practice and has many advantages. It is worth learning. With good methods and with practice one should be able to count one hundred cells in three minutes or less time.

Somewhat more accurate figures can be obtained by counting several separate hundred cells and taking the average of all the counts, but much more depends upon properly made spreads than upon counting large numbers of cells. Counts of cells on a poor spread in which the larger cells have been dragged off do not show the proportion of the cells in the original blood. The student should count several separate hundreds on the same spread and on different spreads of the same blood and see for himself how much they vary. When the count is carefully made on a properly made spread and in the part of the spread which was made before there was any dragging of cells whatever, there is very little variation indeed. In practice, therefore, a properly made count of one hundred cells is sufficient for practical purposes.

Interpretation of variation in the proportion of the normal leukocytes in the blood. The percentage of the different cells in the blood of normal adults should be memorized and should be "at your tongue's end" at all times. It is as follows:

S—20 to 30 per cent
L— 1 to 8 per cent
N—60 to 70 per cent
E— 1 to 4 per cent
B— to 1 per cent

In children under five years of age there are usually from 40 to 50 per cent small mononuclear leukocytes. Often there are even more in very young children. The percentage of neutrophiles is correspondingly less. With increased age the proportions gradually change toward those of the cells in adult blood which are reached soon after puberty. Most women have a little larger percentage of small mononuclear cells and a correspondingly smaller percentage of neutrophiles. It is not at all uncommon to find these cells to be more than 30 per cent of all of the leukocytes in the blood of per-

fectly well women. The small mononuclear leukocytes are increased in those diseases in which there is irritation of the lymph tissues of the body, and especially those in which the disease is fought or resisted largely by this kind of cell. Syphilis, tuberculosis, typhoid and pertussis are examples of such diseases, in all of which there is more or less increase of the per cent of the small mononuclear leukocytes present. They are greatly increased in lymphatic leukemia.

The large mononuclear leukocytes vary so much in health and also under influences not now recognized that slight variations have no special significance. They are greatly increased in acute lymphatic leukemia and in acute infectious mononucleosis.

The neutrophiles are the phagocyters of pyogenic bacteria and their number in the blood is increased in the presence of any disease caused by pyogenic bacteria, provided, of course, nature is able to respond to the call and furnish them. When pyogenic bacteria are present in a tissue from which drainage is poor or impossible and their toxins are absorbed, the neutrophiles are greatly increased; but when there is good drainage, and therefore little or no absorption of toxin into the system, there is little or no increase of the neutrophiles. For instance, staphylococci causing appendicitis or peritonitis would give rise to a very high neutrophile count, while the same bacteria in a skin lesion or other external lesion would give rise to little or no increase; gonococci in a joint give rise to a high count, while there occurs little or no increase in simple infection of the urethra with the same bacteria; streptococci in a middle ear abscess give rise to great increase of neutrophiles before drainage is established, but afterward they fall to near or quite normal in a few hours. An increase in the per cent of neutrophiles indicates, therefore, absorption of toxin from a pyogenic infection or disease in some part of the body, but cannot point out the tissue involved or the particular bacteria concerned. All other things being equal, the less drainage, the more absorption and the higher the count. A very small focus of disease in a tissue from which there is no drainage, like, for instance, in bone, under periosteum, in the middle ear, etc., may give rise to a high neutrophile count. A high neutrophile count indicates, therefore, not only a disease process due to pyogenic

bacteria, but also that drainage is poor or lacking. More than ninety-five per cent of neutrophiles is rarely seen in septic disease.

The neutrophiles are reduced or at least not increased in diseases due to non-pyogenic bacteria, or those not due to bacteria of any kind. Of the common pyogenic bacteria we may mention pneumococci, streptococci, meningococci, staphylococci of almost all kinds, and gonococci. Of the common disease-producing organisms that are non-pyogenic may be named malaria plasmodia, protozoa, spirochetes, typhoid bacilli, tubercle bacilli (unless in very large numbers), and tetanus bacilli.

The eosinophiles are usually increased in intestinal parasite infections, especially hookworm infection, in which they sometimes reach 15 to 20 per cent or more. They are increased in trichinosis sometimes to 75 per cent or more. There is usually great increase during and soon after an attack of true bronchial asthma. There is slight to considerable increase in a variety of parasitic skin diseases. Eosinophilia also occurs in most cases of foreign protein poisoning and anaphylaxis.

The eosinophiles are decreased by pyogenic infection and frequently none are found. An increase of the neutrophiles with marked decrease of the eosinophiles is known as "Simon's septic factor" and is a very strong indication of pyogenic disease or sepsis.

The basophiles are increased to large proportions in some cases of myelogenous leukemia.

For further information as to the differential leukocyte count in certain diseases, see the table on page 50.

Description of abnormal or pathological leukocytes. The neutrophiles, eosinophiles and basophiles found in normal blood are the descendants of myelocytes which are normally present in bone marrow and other blood-making tissues of the body. In certain diseases, notably myelogenous leukemia, myelocytes in large numbers appear in the circulating blood. Within certain limitations most of them can be grouped under one or the other of the following three headings:

A. Neutrophilic myelocytes. These (Plates II and III) are large cells, having a single round, or oval-shaped nucleus, and neutro-

philic granules, which are like the granules in polymorphonuclear neutrophiles, in the cytoplasm. The cytoplasm sometimes takes more or less of the blue in Wright's stain.

B. Eosinophilic myelocytes. These (Plates II and III) are large cells having a single round or oval nucleus, and eosinophilic granules in the cytoplasm, which are like the granules in polymorphonuclear eosinophiles. The cytoplasm usually takes more or less of the blue in Wright's stain.

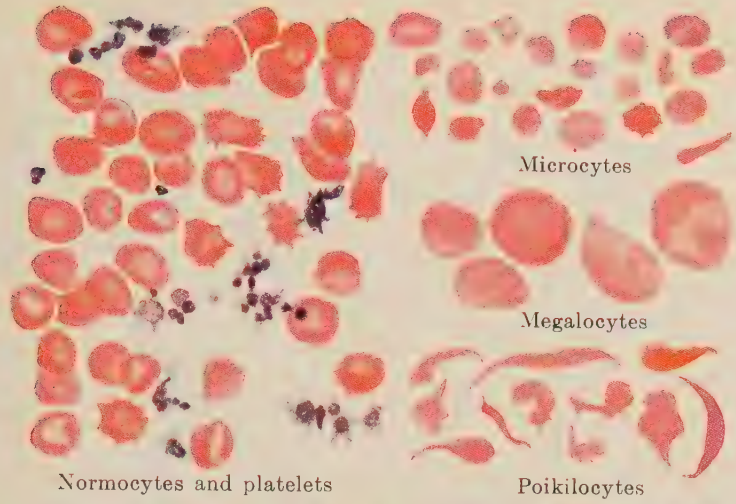
C. Basophilic myelocytes. These (Plate III) are large cells having a single round or oval nucleus and basophilic granules in the cytoplasm, which are like the granules in polymorphonuclear basophiles.

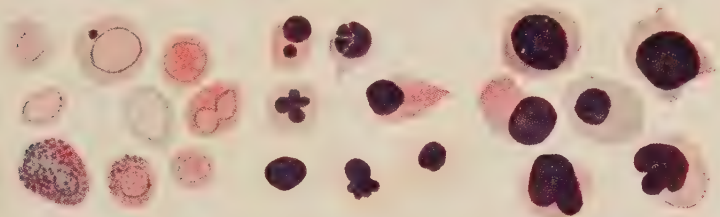
It should be understood that the myelocytes are simply young granulocytes that have appeared in the circulating blood before they had aged into the adult polymorphonuclear cells, and there must therefore be a good many cells in such a blood that are on the border line (Plates I and II) between them and it is difficult to know whether to put some of these down as myelocytes or as normal leukocytes.

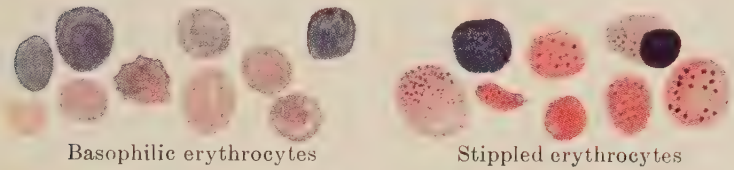
In addition to the abnormal leukocytes which fall in one or the other of the above groups, there are usually a good many others in leukemic blood that cannot be classified. They should all be grouped under the head of unclassified cells. When making a differential leukocyte count and myelocytes are found present, one should add to the usual tally sheet, a heading for each variety, as, for instance, MN, ME and MB.

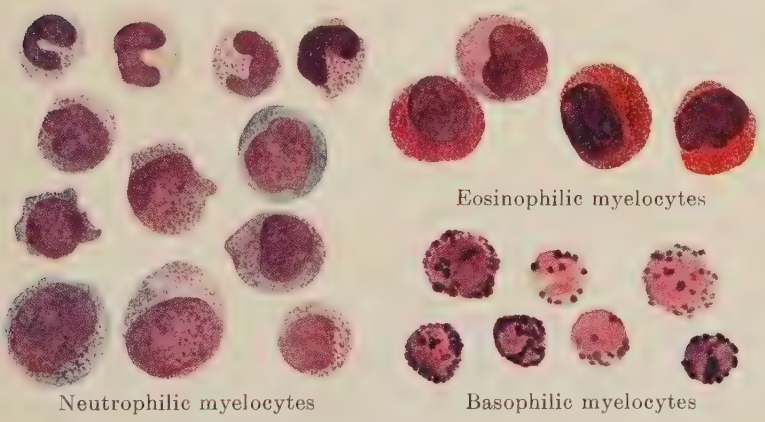
Interpretation of the presence of pathological leukocytes. Myelocytes (approaching the adult polymorphonuclear neutrophile cell type) may occasionally be found in small numbers in blood in which there is a considerable septic leukocytosis and sometimes even when there is no leukocytosis. They should be counted in and considered with the neutrophiles. Large numbers are not found except in some form of myelogenous leukemia. Myelocytes of one or more different kinds are present in large numbers, usually 75 to 90 per cent of all the cells, in all cases of myelogenous leukemia.

The mononuclear cells, small and large, are greatly increased

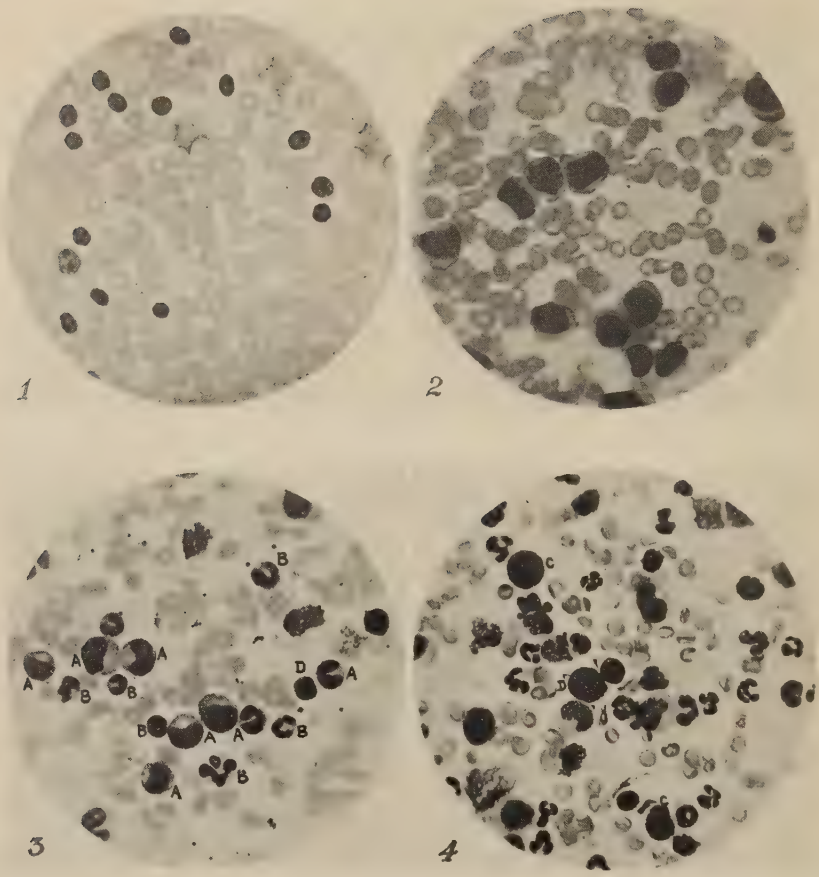








PATHOLOGICAL BLOOD CELLS



PHOTOMICROGRAPHS OF BLOOD IN DIFFERENT TYPES OF LEUKEMIA

1. Chronic lymphatic leukemia. Normal appearing small mononuclear leukocytes predominating. Note the numerous ruptured cells.

2. Acute leukemia. Large mononuclear cells some of which are granular. Note normoblast.

3 and 4. Chronic myelogenous leukemia. *A*, neutrophilic myelocytes; *B*, completely differentiated or adult polymorphonuclear neutrophiles; *C*, eosinophilic myelocyte; *D*, nucleated erythrocytes stained very deeply and photographing black.

(Plate III) in lymphatic leukemia. Percentages from 75 to 90 and above are often reached. It is not uncommon to find atypical cases of leukemia in which there are a great many cells that cannot be classified satisfactorily.

CHAPTER IV

Blood—*continued*

TOTAL BLOOD CELL COUNTS, LEUKOCYTES, ERYTHROCYTES, PLATELETS; HEMOGLOBIN ESTIMATION

Total leukocyte counts. Special apparatus and material required. The special apparatus required are: A. Diluting pipette (Fig. 37). This should provide for diluting the blood one hundred times. B. Counting chamber (Fig. 38). The counting chamber devised by one of us (Bass) and improved by the other (Johns) so as to better adapt it to counting the cells in spinal fluid as well as blood has advantages over all others. The chief advantage lies in the sim-

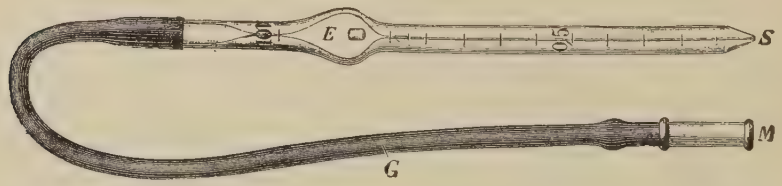


Fig. 37. Diluting pipette.

plicity of the ruling. This same Bass-Johns ruling is now supplied on the several different styles of counting chambers. It consists of a heavy object slide (Fig. 39) with a central rectangular ruled counting plate and a raised plate on either side of it, extending across the slide, each separated by grooves. The two raised plates are exactly 0.1 mm. higher than the counting plate, so that when a cover-glass is placed over both there will be a space of 0.1 mm. between it and the counting plate.

The original Bass ruling for counting blood (Fig. 40) in the middle of the counting plate consists of a square of 2 mm. side. This is divided into ten rectangles, 0.2 mm. by 2 mm., for counting leukocytes. For counting erythrocytes there are superimposed upon the

ruled space at each corner and in the center five squares of 0.2 mm. side, each further divided into 16 small squares of 0.05 mm. side. The cubic contents of the entire ruled space, 0.1 mm. deep, is 0.4 cu. mm. The total cubic content of the five large squares is 0.02 cu. mm.

In the improved ruling there are 15 rectangles added of the same dimensions as the original 10, making a total of 25 and giving a total content of 1 cu. mm. The entire ruled area is counted in counting spinal fluid where there are very few cells. It may all be

Fig. 38. Counting chamber, Bass-Johns ruling.

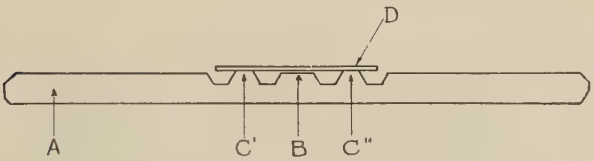


Fig. 39. Counting chamber. Side view.

counted in counting leukocytes, thereby increasing the accuracy slightly.

In addition to this special apparatus, a diluting fluid is required. We use Toison's fluid which permits counting both leukocytes and erythrocytes in the same preparation. (Formula 2.) It should be kept in bulk in a tall, narrow bottle and whenever a count is to be made, enough of the solution should be poured off (into a small bottle) for present use. *Do not use directly from stock bottle. Do not return unused solution to stock bottle.* If managed as directed, the solution usually keeps well; otherwise, it may deteriorate from the growth of moulds and yeasts and soon become unsatisfactory.

For counting leukocytes only, a 2 per cent solution of acetic acid in water, which destroys the erythrocytes and makes the leukocytes easier to see, is useful. It can be made up extemporaneously when required for use, or it may be poured from a stock bottle when required as advised above for Toison's solution. An 0.85 per cent sodium chloride solution ("normal saline") is a fairly good diluting fluid for either or both erythrocytes and leukocytes.

Cleaning the pipette and making the dilution. The pipette must be clean and perfectly dry before use. Assuming that it has been

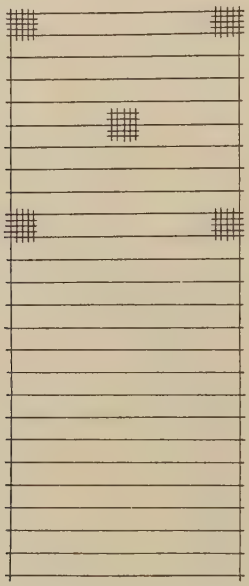


Fig. 40. Bass-Johns ruling.

Fig. 41. Chapman pump.

used it is cleaned and dried as follows: (1) Slip off the rubber tube and blow through the tip of the pipette to expel any fluid present. (2) Replace the rubber tube and draw pipette full of water. (3) Slip off rubber tube and blow out water. (4) Replace rubber tube and draw pipette full of alcohol. (5) Slip off rubber tube and blow out alcohol. (6) Replace rubber tube and draw pipette full of ether. (7) Slip off rubber tube and remove ether by slinging the pipette somewhat like "shaking down" the mercury in a thermometer. (8) Replace rubber tube and remove last trace of ether by sucking air through the pipette when it will be dry and ready for

use. Always clean and dry the pipette after use and have it ready for the next time. When thoroughly dry the bead in the bulb of the pipette rolls about freely when the pipette is shaken. A Chap-

FIG. 42. Taking up blood into diluting pipette from patient's finger. Note that the operator's right hand is steadied by touching a finger against the finger of the patient.

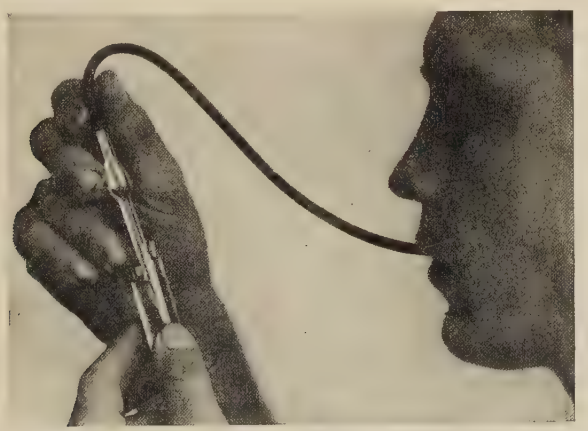


FIG. 43. Filling diluting pipette with diluting fluid. Pipette held high enough so that the marks are level with and are plainly visible to the eye.

man (Fig. 41) suction pump with which to draw the cleaning fluids and air through the pipette is convenient and useful if many pipettes are to be cleaned.

To make the dilution, have the diluting fluid ready. Well up a good sized drop of blood (without squeezing hard) and draw blood (Fig. 42) into the pipette to the mark 1. Quickly wipe the tip of the pipette to remove any blood on it and draw up (Fig. 43) diluting fluid until the mixture reaches the mark 101, being careful not to get an air bubble in the pipette. Now revolve the pipette (Fig. 44) to mix thoroughly and the dilution is ready for use. The counting may be done any time within an hour or two. If to be carried any

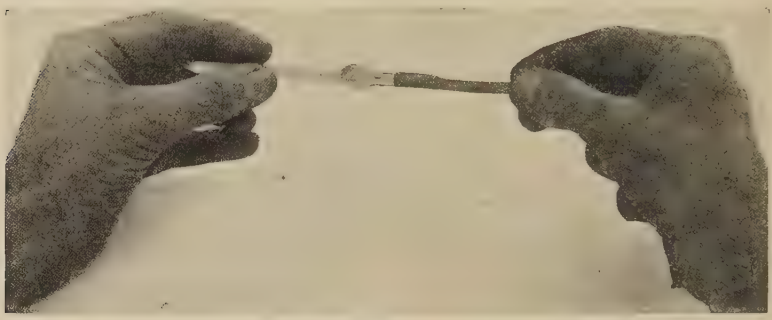


Fig. 44. Revolving pipette to mix contents. Note that pipette is held loosely in the left hand at about the middle of the stem. By holding the rubber tube as shown and revolving it with the fingers of the right hand, rotary jarring motion and complete mixing of the blood and diluting fluid is secured.

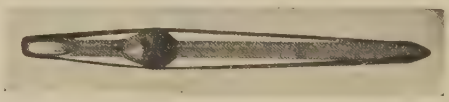


Fig. 45. Pipette containing diluted blood. Rubber band stretched over ends to prevent escape of fluid when carrying.

considerable distance to the laboratory, stretch a rubber band over the ends of the pipette (Fig. 45) to prevent the fluid from running out. Lay pipette on side and do not allow it to stand on end for any length of time.

Making the preparation. The counting chamber and cover-glass must be scrupulously clean and free from dust particles. Wash and carefully dry the counting chamber and then wipe it free of dust particles with a handkerchief or similar piece of cloth. If it is held so that the light falls upon it at an angle and so that there is a dark

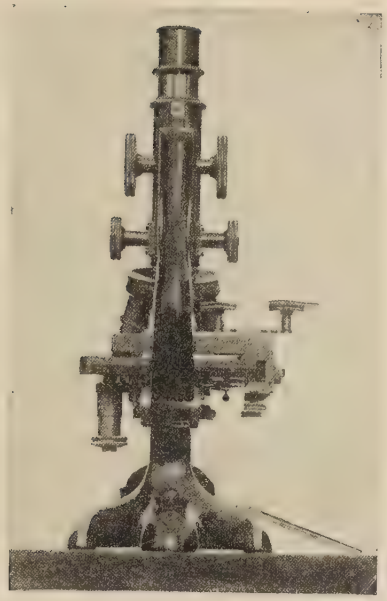


FIG. 46. Counting chamber after it has been cleaned, resting face downward against the foot of the microsope. Cleaned cover-glass resting upon the wheel of the mechanical stage.

FIG. 47. Cleaning cover-glass. Note that it is held by the edges only. The cleaned surface must not be touched by the fingers.

background in the distance, dust particles can be seen much better. These should be picked off with the handkerchief wrapped around the end of the finger. Having it clean, rest it face downward against some convenient object, as for instance, the foot of the microscope (Fig. 46), to prevent dust particles from settling on it before use.

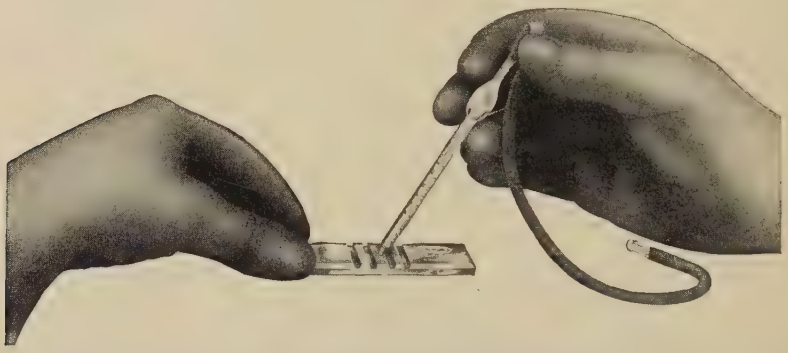


FIG. 48. Squeezing out diluted blood upon the counting plate. Note folding of soft rubber tube and manner of holding the pipette.

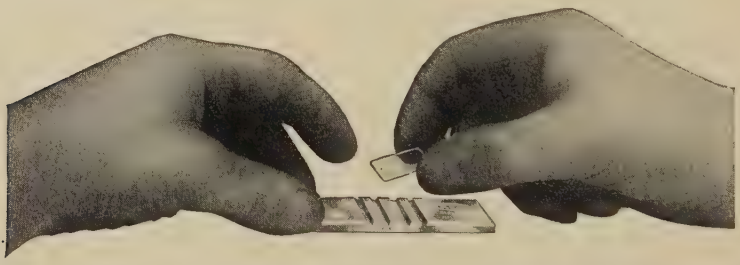


FIG. 49. First step in placing cover-glass upon the counting chamber. Note that both hands rest (to steady them) upon the table.

Now thoroughly clean the cover-glass. Wash it if necessary. Handle by the edges only (Fig. 47). After it has been wiped as clean and free of dust particles as possible, with the handkerchief, hold it in proper indirect light with a dark background in the distance to see any remaining dust particles which should then be picked off. Now place it, clean side down, upon some convenient dust free surface, like the wheel of the mechanical stage (Fig. 46), until required for use.

Having the counting chamber and cover-glass ready for use, have convenient some hard glass or metal object (preferably not sharp on the edge) with which to press down the cover-glass when needed. The handle of the platinum loop or eye end of the blood sticker needle or even the homeo vial which holds it are convenient

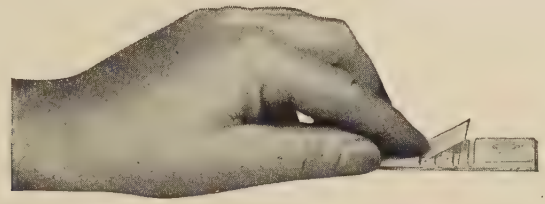


FIG. 50. Second step. The cover-glass is tilted up by the pressure of the finger upon the edge that extends slightly into the groove.

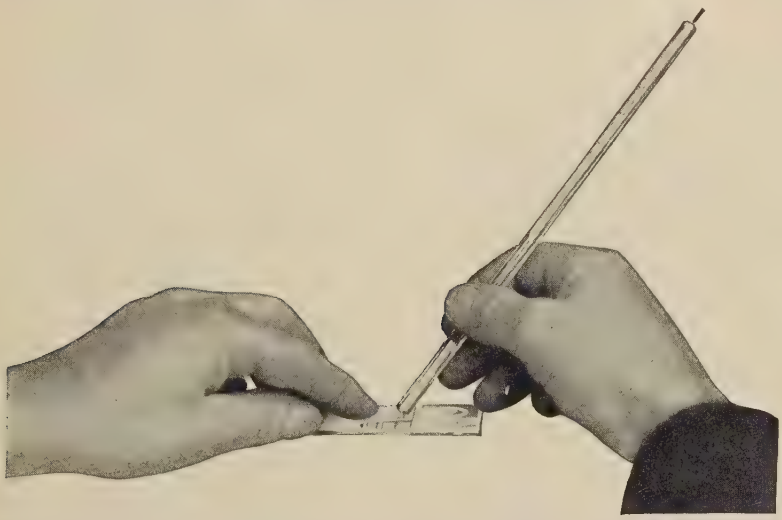


FIG. 51. Third step. Bringing down the cover-glass by pressure with the handle of the platinum loop (or other suitable hard instrument).

for this purpose. Wood or rubber instruments are not good, nor is a lead pencil.

Now thoroughly mix the contents of the pipette (rubber tube attached) and blow out two or three drops of fluid to wash out the long arm of the pipette. Then quickly squeeze (Fig. 48) onto the

center of the counting plate the necessary amount of the diluted blood. A little experience is necessary to get the proper amount and to perform the manipulation quickly. There should never be sufficient to run over into the moat, but it must always more than cover the ruled area. Place the cover-glass over it at once. The steps in doing this properly are illustrated in Figs. 49, 50, 51, 52. The cover-glass should now be pressed down (Fig. 53) firmly at each

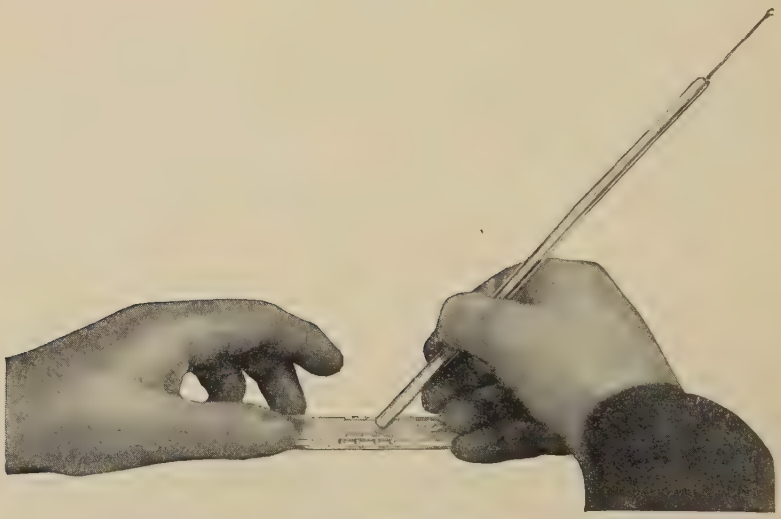


Fig. 52. Fourth step. The cover-glass has been brought down and the finger has been lifted from it.

of the four corners. Do not slide the cover-glass in doing this. There must never be fluid or dust particles between the cover-glass and the plates on which it rests. The directions sometimes given by manufacturers of the new style counting chambers to allow the fluid to flow under the cover-glass is a mistake and should not be followed.

If the surfaces of the plates and cover-glass are perfectly clean and free from dust particles, and if the manipulation has been properly done, Newton's color rings (rainbow colors) will be present on each side. These can be seen best by looking at the top of the preparation held toward the light of a window or door or an artificial

light and just a little below the level of the eyes. Unless they are present the preparation is not a good one. *Do not tilt the preparation. Hold it level.* Now lay it down for two or three minutes during which time all the cells settle onto the surface of the counting plate.

In spite of good technic and usually due to slow manipulation, the cells sometimes settle unevenly and the next step is to examine

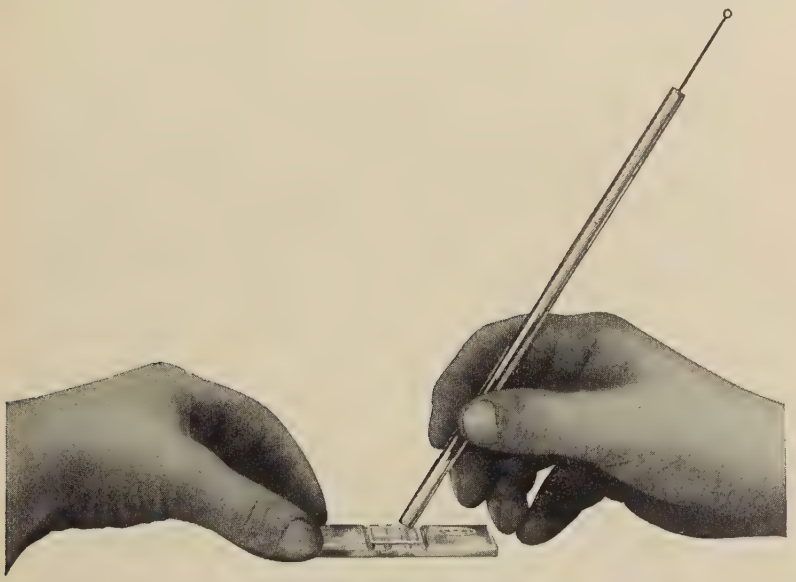


FIG. 53. Fifth step. Pressing down the cover-glass at each corner. Note that the hand holding the instrument with which pressure is made is steadied by resting upon the table. Care must be taken to avoid *slipping* the cover-glass.

the preparation to determine this question. Hold it (level) above the level of the eyes (Fig. 54), having the light fall upon it from above, but look at it from below with a dark background in the distance. The evenness (Fig. 55) or unevenness (Fig. 56) can be seen, and if the cells are not evenly distributed the preparation is not good.

Counting the cells. Reduce and adjust the light of the microscope so that the ruling can be seen best. Count with the 4 mm. objective. Find the upper left hand corner of the ruled area (in fact the lower

right hand corner; images are reversed in the microscope). Place the end of the first rectangle in the center of the field and move toward the right, counting all the leukocytes found in the rectangle. When the end is reached drop down until the next rectangle is in the center of the field and move in the opposite direction until all the cells in it are counted. Drop down to the next and count all the leukocytes in it in the same way. Thus continue until all the leukocytes in the 10 rectangles have been counted (or all in the 25, if you count the entire ruled area). Make the rule of counting *in*

FIG. 54. Looking at distribution of cells upon ruled plate. Dark background directly in front of and a few feet away. Light from window at left.

any cell found upon the left or upper boundary line of any rectangle when counting the cells in that area, but count *out* any cell found upon the right and lower boundary lines of a given rectangle, when counting the cells in it.

The leukocytes are stained violet when Toison's fluid is used. They are clear, many-shaped, prominently appearing bodies when acetic acid is used. The small ruling is disregarded when counting the leukocytes.

Multiply the number of leukocytes counted in the first ten

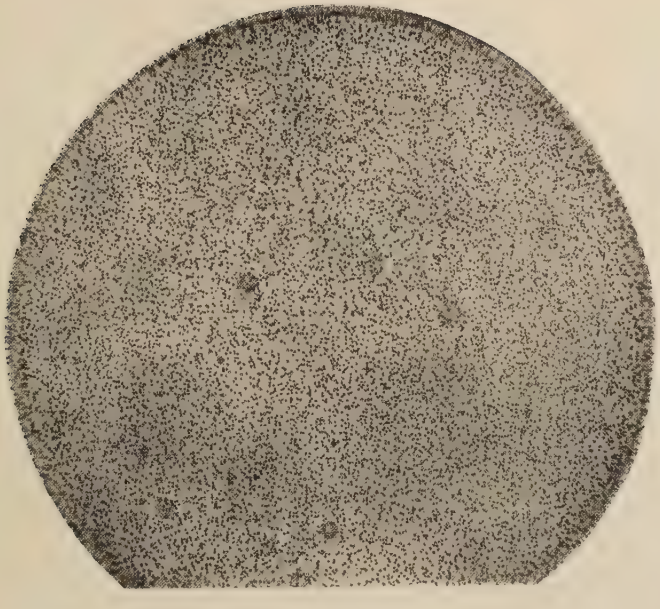


FIG. 55. Even distribution of cells upon the ruled plate.

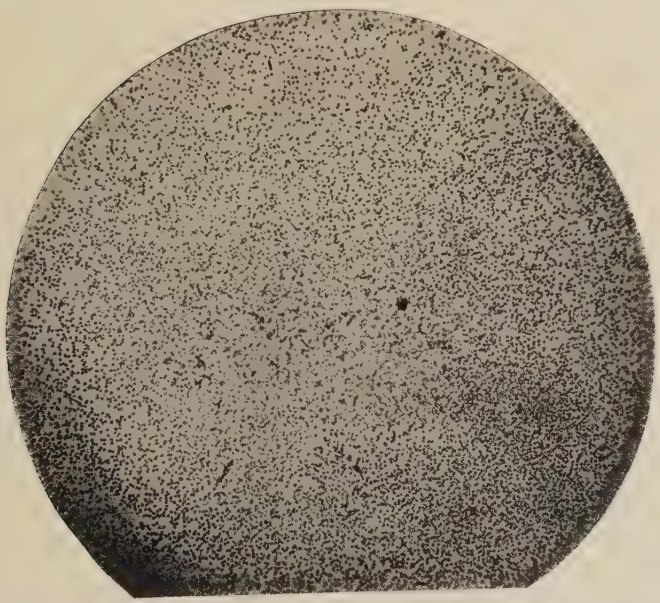


FIG. 56. Uneven distribution of cells. Note thin area at top and thick area on the right.

TABLE INDICATING THE TENDENCY OF THE TOTAL AND DIFFERENTIAL LEUCOCYTE COUNTS IN SOME OF THE COMMON DISEASES AND CONDITIONS

It should be understood that only the tendency can be stated and that no hard and fast invariable rules can be laid down. It should also be understood that any increase or decrease of the percentage of any variety of leucocytes present naturally has the reverse effect upon the percentage of all the other cells, although the actual total number of the latter may not be effected. Blanks in the table indicate no special change.

DISEASE OR CONDITION	TOTAL LEUCOCYTE COUNT	DIFFERENTIAL LEUCOCYTE COUNT				
		Small mononuclears	Large mononuclears	Neutrophiles	Eosinophiles	Basophiles
Actinomycosis	Increase					
Addison's disease						
Anemia (pernicious)	Decrease or normal			Decrease	Decrease	Decrease
Anemia (post-hemorrhagic)	Increase			Increase		
Appendicitis (acute)	Increase, usually great			Increase	Decrease	
Appendicitis (chronic)	Increase, moderate or slight			Increase, slight		
Arthritis (acute)	Increase, usually great			Increase	Decrease	
Asthma (bronchial, during attack)	Increase, slight or moderate				Increase	
Bronchiectasis						
Bronchitis (acute)	Increase, slight or moderate			Increase		

Bronchitis (chronic)						
Burns (extensive)	Increase, usually great, occurring promptly			Increase		
Carcinoma	Increase may occur in advanced cases					
Chlorosis						
Cholangitis	Increase, usually great			Increase		
Cholecystitis	Increase, great during acute stage			Increase		
Cholelithiasis (colic) attack						
Cholera (asiatic)	Increase			Increase, slight		
Cirrhosis of liver						
Cystitis (acute)	Increase			Increase		
Dengue	Decrease	Increase	Increase	Decrease		
Diabetes mellitus						
Digestion (heavy meal)	Increase, slight					
Diphtheria	Increase, moderate; greater in severe cases			Increase		

TABLE INDICATING THE TENDENCY OF THE TOTAL AND DIFFERENTIAL LEUCOCYTE COUNTS IN SOME OF THE COMMON DISEASES AND CONDITIONS—Continued

DISEASE OR CONDITION	TOTAL LEUCOCYTE COUNT	DIFFERENTIAL LEUCOCYTE COUNT				
		Small mononuclears	Large mononuclears	Neutrophiles	Eosinophiles	Basophiles
Eclampsia (puerperal)	Increase					
Endocarditis (ulcerative)	Increase, moderate or great			Increase	Decrease	
Erysipelas	Increase, usually great			Increase	Decrease	
Filariasis	Increase, slight when heavy infection				Increase, usually great	
Gastritis (acute)	Increase, slight or moderate			Increase		
Gastritis (chronic)						
Gonorrhea						
Gonorrheal: Arthritis Epididymitis Orchitis, etc.	Increase			Increase		
Gout						
Helminthiasis Hookworm Strongyloides Tricocephalus Oxyuris Ascaris Tenia					Increase in most cases. Highest in the order named	

Hepatitis (liver abscess)	Increase during active stage			Increase		
Hodgkin's disease	Increase, late			Increase, slight	Increase	
Influenza	Decrease or normal	Increase		Decrease		
Intestinal obstruction	Increase, usually 20,000 or more			Increase	Decrease	
Kala-azar			Increase, usually great			
Leukemia (acute lymphatic)	Increase great, often to 100,000 or 200,000	Increase	Increase very great			
Leukemia (chronic lymphatic)	Increase great, often to 300,000 or 400,000	Increase very great. May reach 90 per cent	Increase			
Leukemia (myelocytic)	Increase enormous, often to 500,000 or more			Increase	Increase	Increase
				Corresponding myelocytes present in large numbers		
Malaria			Increase			
Measles (rubeola and rubella)						
Meningitis (cerebrospinal)	Increase great in all forms except tubercular, where increase is moderate or slight in most cases	Increase in tubercular		Increase	Decrease	

TABLE INDICATING THE TENDENCY OF THE TOTAL AND DIFFERENTIAL LEUCOCYTE COUNTS IN SOME OF THE COMMON DISEASES AND CONDITIONS—Concluded

DISEASE OR CONDITION	TOTAL LEUCOCYTE COUNT	DIFFERENTIAL LEUCOCYTE COUNT				
		Small mononuclears	Large mononuclears	Neutrophiles	Eosinophiles	Basophiles
Mumps		Increase				
Myxedema						
Nephritis (acute)	Increase, usually slight or moderate			Increase	Decrease	
Nephritis (chronic parenchymatous)	Increase, usually slight			Increase, slight	Decrease, slight	
Otitis media and mastoiditis	Increase in all acute cases			Increase	Decrease	
Pellagra		Slight increase				
Pneumonia	Increase, often to 30,000 or 40,000			Increase	Decrease	
Pregnancy	Increase, slight or moderate during last 3 or 4 months					
Pyelitis and pyelonephritis	Increase			Increase		
Sarcomatosis	Increase in advanced stage					
Scarlatina	Increase, usually great			Increase	Increase late	
Scurvy	Increase					

Septicemia due to staphylococci, streptococci and pneumococci	Increase in all cases			Increase, great	Decrease	
Smallpox	Increase only moderate or not at all until pustulation, when, moderate increase occurs			Increase during pustular stage		
Syphilis	Increase, slight during secondary stage	Increase				
Tonsillitis (acute)	Increase			Increase	Decrease	
Trichinosis	Increase, slight				Increase great	
Tuberculosis (acute miliary)	Increase	Increase	Increase			
Tuberculosis (pulmonary)	Increase in advanced stage, due to secondary infection	Increase		Increase in advanced stage		
Typhoid and paratyphoid	Decrease	Increase				
Typhus fever						
Uremia	Increase, usually moderate					
Whooping cough	Increase moderate during active stage	Increase great			Increase	
Yellow fever						

rectangles by 250 (or the number counted in all 25 rectangles by 100). This gives the number of cells per cubic millimeter.

Interpretation of total leukocyte counts. Blood of normal individuals contains 5,000 to 8,000 leukocytes. There is a slight increase during digestion and exertion, and children's blood gives slightly higher values than adults'.

The total number of leukocytes is increased in leukemia sometimes to very high figures. Seven hundred thousand has been recorded in myelogenous leukemia, but 400,000 to 500,000 is an average high count. In lymphatic leukemia the count usually ranges between 100,000 and 400,000 leukocytes.

The total number of leukocytes is increased in those diseases in which there is increase in the percentage of particular cells as a result of irritation of the tissue which produces them, or as a result of the demands of nature for the particular cells to resist the disease process present. For instance, there is usually slight increase in syphilis during the stage of lymph tissue involvement. In the worst cases of disease due to pyogenic bacteria and in which much toxin is absorbed, the total leukocyte count may be increased to 40,000, or even 50,000, but these figures are rare. Naturally the count depends not only upon the disease or toxin which calls out the cells, but also upon the capacity of the individual to meet the demand. Long-continued disease often tends to exhaust the capacity to respond.

On pages 50 to 55 we give a list of the common diseases and their influences upon the differential and total leukocyte count. It should be understood that this gives only the general tendency and cannot be exactly correct in all cases, and in all combinations of diseases. The total leukocyte count should be interpreted in the light of the other clinical facts in the case as should also the differential leukocyte count.

Counting the erythrocytes. The same apparatus is used as in making total leukocyte counts. Use either Toison's fluid or 0.85 per cent salt solution as diluting fluid. Dilute the blood one hundred times. When Toison's fluid or salt solution is used, the leukocytes and erythrocytes may be counted in the same preparation. In either case make the preparation in the counting chamber as described for counting leukocytes.

In the Bass-Johns ruling (Fig. 40) there are five large squares,—one at either end of the first and tenth ruled rectangles and one in

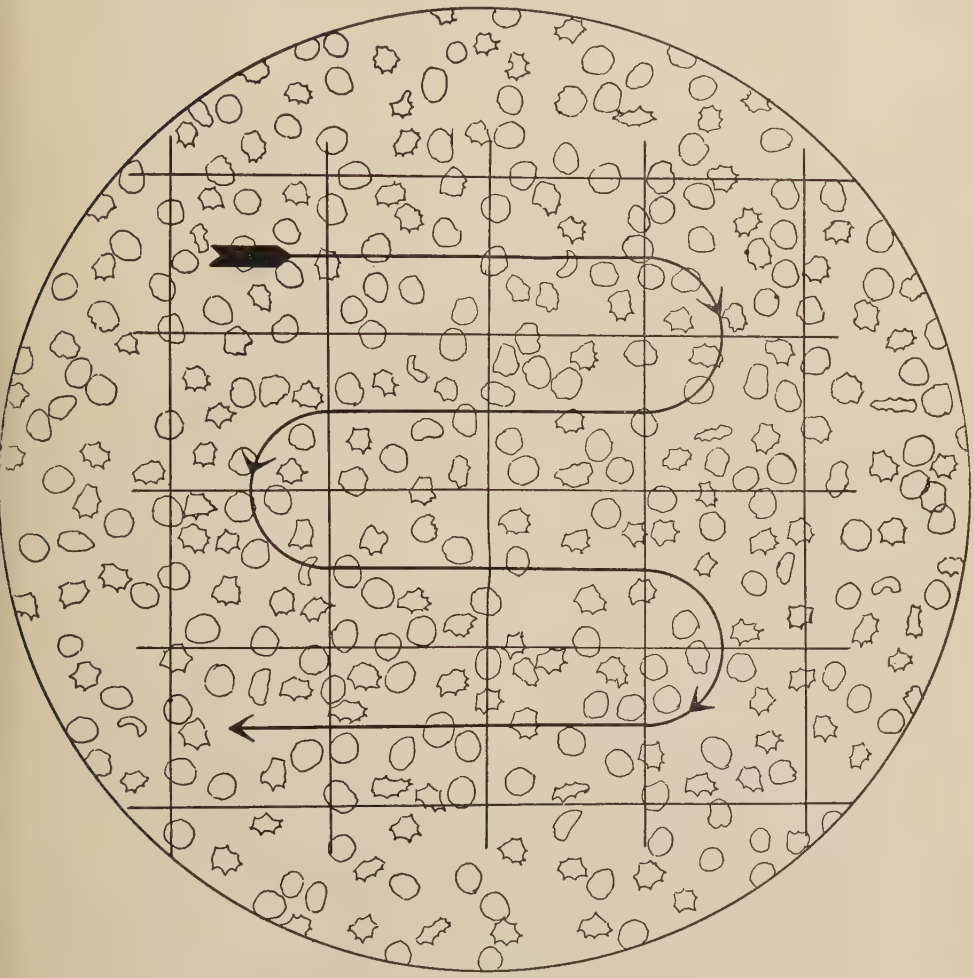


Fig. 57. Arrow indicates order in which the erythrocytes in the sixteen small squares in one large square should be counted. We count 200 cells here.

the middle of the fifth and sixth, each ruled into sixteen small squares. Count the erythrocytes in each of these large squares. Begin at the upper left hand corner and count all the erythrocytes in one small

square after another (Fig. 57) until all are counted. Much time can be saved by counting by twos or fives instead of by ones. When considering any given ruled area count *in* all cells that touch the left or upper lines (Fig. 58) and count *out* all that touch the right and lower lines. Do not stop to write down the cells until all in a large square have been counted. Add up the number of cells counted in the five large squares. The total number of cells counted in the five large squares multiplied by 5,000 equals the number of cells per cubic millimeter.

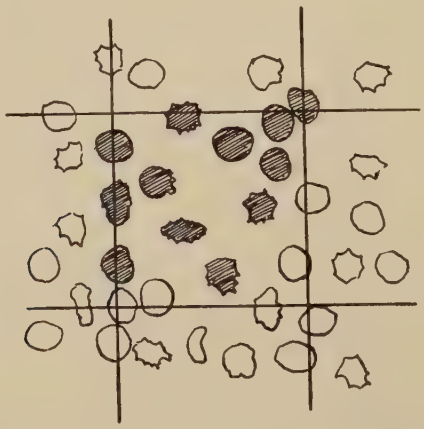


FIG. 58. The twelve cells to be counted in this small square are shaded. Those not shaded are not counted.

Estimation of the hemoglobin. Hemoglobin percentage may be estimated only crudely and approximately with the Tallquist scale. This method is given here because of its extreme simplicity and practibility, notwithstanding its inaccuracy. The Tallquist scale consists of a series of ten red colored cards (Plate IV), each representing different percentages of hemoglobin in steps of ten and ranging from 10 to 100 per cent. The colored cards are mounted on one sheet and bound in book form, with several pages of soft white filter paper. Each colored card is perforated in the middle with a hole about one-fourth inch in diameter.

Well up a good drop of blood without much squeezing and touch a piece of the white paper to it. A spot on the paper is thus satu-

TALLQUIST HEMOGLOBIN SCALE

The colors here shown are not exactly the same shade as in the original, they do not correspond to the different percentages of hemoglobin and cannot be used to ascertain or measure the quantity of hemoglobin.

rated with blood. Wait just a moment to allow the blood to spread through the paper by capillarity as far as it will and then promptly compare it with the different shades of the color sheet, holding the paper behind and viewing the blood through the holes in the color card. Pass quickly from one to the other until the blood is matched with a shade on the color sheet and note what per cent it represents. This is the estimate of percentage of hemoglobin in the blood. Accuracy improves with practice. Sometimes the color seems to fall between two cards or shades, and in such case the estimate should be made accordingly. For instance, if it seems to fall between 80 and 90 per cent we would call it 85 per cent.

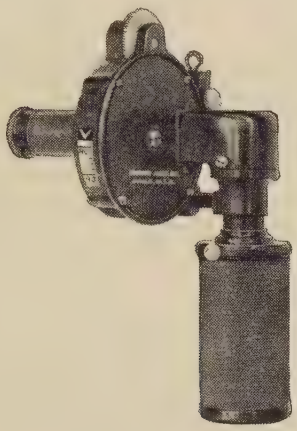


FIG. 59. Improved Dare hemoglobinometer with telescoping camera and flashlight. Rieker Instrument Company, Philadelphia.

More accurate estimation of hemoglobin is obtained with the Dare hemoglobinometer. It consists essentially of an automatic pipette for collecting the blood between two glass plates and a graduated color scale with a corresponding colored glass plate enclosed in a dust-proof, hard rubber case containing also a small telescoping camera tube. The latest improved and best type (Fig. 59) is (No. 1011) made by Rieker Instrument Company, Philadelphia, and is especially recommended. It contains a suitable electric flash light. As the reading should be made very promptly after the blood is drawn, the apparatus should be gotten ready in advance. Stick the patient's finger or ear, well up a large drop of blood, fill

the pipette by touching it to the blood and allowing it to fill by capillary force, place it in the instrument, turn on the light and match, turning the disc as necessary and read off the per cent indicated. Make another reading or two and take the average.

Color index. The color index of blood is an expression of the average amount of hemoglobin per erythrocyte, the normal being represented by 1. Divide the percentage of hemoglobin by the percentage of erythrocytes. The quotient is the color index. A quick way to find the per cent of erythrocytes is to multiply the first two figures on the left hand side of the number of erythrocytes per cubic millimeter by 2, unless it is less than one million, when only the first figure would be multiplied by 2.

Interpretation of number of erythrocytes, hemoglobin per cent and color index. In health, females have about 4,500,000 to 5,000,000 erythrocytes per cubic millimeter, and males have about 5,000,000 to 5,500,000. Five million is generally considered the average, but healthy, vigorous men will average nearer 5,500,000.

The hemoglobin in blood of normal individuals is about 100 per cent, but there is a slight variation above, or especially below, in most people. Reading with the Tallquist scale or the Dare instrument, we may consider values of 80 to 110 per cent within the bounds of technical variation and the variation of normal blood.

The color index of the blood of normal individuals is theoretically 1, but there is a slight variation above or below this in apparently healthy individuals. Indices of from 0.8 to 1.15 are within the limits of the variation in the blood of normal individuals and of technical variation.

The number of erythrocytes may be increased by any disease or influence that removes considerable water from the blood, provided, of course, a corresponding amount is not taken in at the same time. Acute diarrhea, profuse sweating, etc., may lead to increase in the total number of cells per cubic millimeter. Abstinence from fluids leads to the same condition. Profuse diuresis, such as occurs in advanced diabetes, usually leads to concentration of the blood cells. Decompensation in various heart conditions tends to concentrate the blood and thereby increase the erythrocyte count. In addition to these various dehydrating influences or diseases which may lead to an increased number of erythrocytes, there is a disease, idio-

pathic polycythemia, in which the total erythrocyte count sometimes reaches as high as from 7,000,000 to even 10,000,000 per cubic millimeter.

The hemoglobin percentage is increased by any of the above influences that increase the erythrocyte count. The color index would therefore be unchanged.

The number of erythrocytes is decreased in all anemias. The decrease may be much or little according to the grade and kind of anemia. Counts as low as 500,000 sometimes, but very rarely, are observed. A count below 1,000,000 should be considered a very severe anemia. The kind of anemia is indicated and the diagnosis much aided by a consideration of both the erythrocyte count and the hemoglobin percentage—the color index.

The number of erythrocytes is reduced in the primary anemias as, for instance, pernicious anemia, to sometimes 1,000,000 cells or less; and although the hemoglobin is also always below normal, the reduction of the hemoglobin is not so great as that of the number of the erythrocytes. This gives rise to a high color index (1.15 to 1.65 or more). A color index well above the limits of technical and normal variation strongly indicates pernicious anemia.

The number of erythrocytes is somewhat reduced in chlorosis, but usually the reduction is only moderate. In fact, a great many cases of chlorosis have more than 4,000,000 erythrocytes. The hemoglobin is greatly reduced in this disease and this gives rise to a very low color index. Such indices as 0.35 to 0.5 are common, and strongly indicate chlorosis.

In secondary type anemia, due to loss of blood or to disease, there is more or less reduction in the number of erythrocytes according to what may be the cause in the particular case. The hemoglobin is reduced more than the number of erythrocytes, giving rise to a low color index. The color index is always below the normal limits in secondary anemia. In long standing secondary anemia with low hemoglobin and erythrocytes the color index may approach or reach normal figures. The lowest color index occurs in chlorosis.

Pathological erythrocytes. Sometimes there is great variation in the size of the erythrocytes. Some are much smaller than the normal and are called microcytes. Others are much larger than the normal size and are called megalocytes. This condition of great variation in the size of the erythrocytes is called *anisocytosis* (Plates II and V).

The shape of normal erythrocytes is that of a biconcave disc. Sometimes there is great variation in the shape of many of the cells. This condition is known as poikilocytosis and such abnormally shaped erythrocytes are *poikilocytes* (Plates II and V).

Normal erythrocytes in the circulating blood never have nuclei. In certain blood diseases, especially the primary anemias, a few to very many nucleated erythrocytes (Plates II and V) are present. The nuclei vary in size. They are round or oval, sometimes presenting two or more lobes and in a good many of the larger ones a reticulated structure can be made out. This gives some suggestion of the spokes of a wheel. Nuclei of erythrocytes stain dark blue to almost black with Wright's stain.

When very large erythrocytes (megalocytes) contain nuclei they are called *megaloblasts* (Plates II and V).

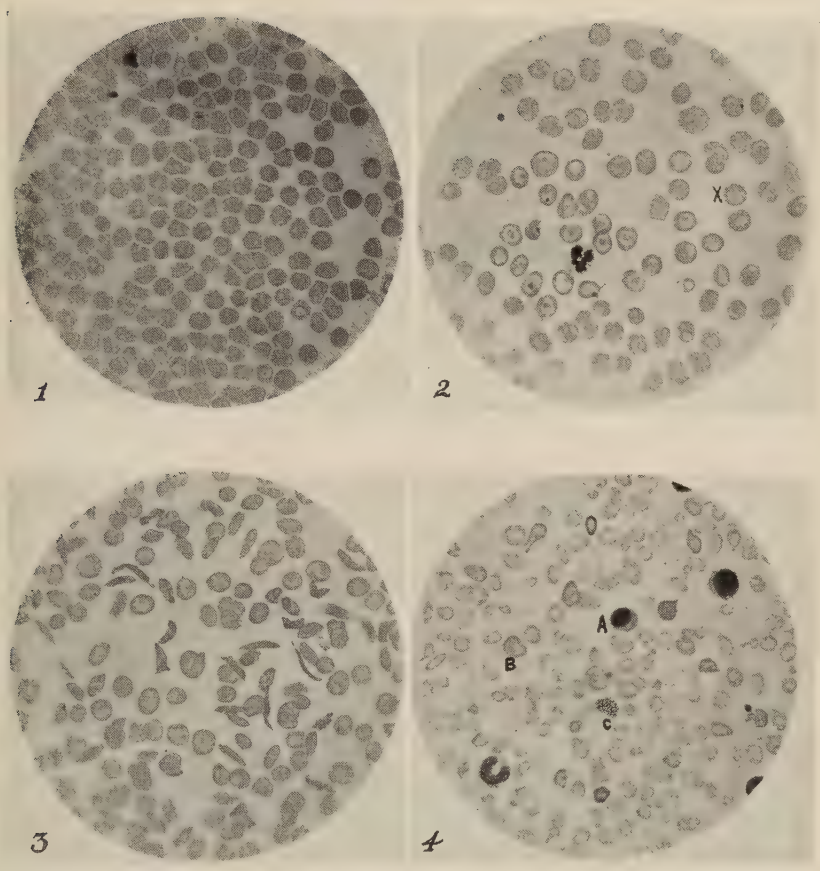
A normal size erythrocyte containing a nucleus is a *normoblast* (Plates II and V). We sometimes find *free nuclei.*

In anemia, due to certain poisons, notably chronic lead poisoning, pernicious anemia, the leukemias, and to a less extent malaria, a few to many of the erythrocytes contain basophilic (blue staining) granules. These vary much in size and number from a few coarse granules to many very fine granules. Such cells are known as *stippled,* or *granular erythrocytes* (Plates II and V).

In normal blood all the erythrocytes take about the same shade of color when stained with Wright's (or other polychrome) stain, but in pathological blood, certain cells are often basophilic and take more or less of the blue. These cells are known as basophilic erythrocytes and the condition of the blood as polychromatophilia (Plates II and V).

Reticulated erythrocytes. These are young erythrocytes which, when stained supravitally with brilliant cresyl blue, show a network of purple granular filaments in the cytoplasm.

Technic. Place in a test-tube about equal quantities of 0.5 per cent solution brilliant cresyl blue in 0.85 NaCl solution and defibrinated blood. Shake tube and let stand 10 minutes, centrifugalize, pour off supernatant fluid, shake up sediment, make spread on slide and stain with Wright's stain. Scratch several parallel lines on specimen with a sharp pointed instrument to serve as guides. Count several hundred cells and note number that are reticulated. Calculate percentage.



PATHOLOGICAL ERYTHROCYTES

1. Normal blood (oil immersion). Very marked changes in shape of normal cells may be produced by surface tension between closely packed cells. This is normal.

2. Pernicious anemia, between "showers." Moderate anisocytosis with slight poikilocytosis. *X*, normal size cell. Numerous megalocytes and a few microcytes.

3. Sickle-cell anemia. Extreme poikilocytosis is shown. Poikilocytosis is always accompanied by anisocytosis.

4. Hemolytic jaundice. Severe anemia: Anisocytosis, poikilocytosis and normoblasts, *A;* polychromatophilia, *B;* and stippling, *C*.

Interpretation. Normal blood contains less than 1 per cent. Increase, sometimes to 15 or 20 per cent, indicates active blood regeneration.

Interpretation of pathological erythrocytes. Nucleated erythrocytes, anisocytosis, poikilocytosis and polychromatophilia occur chiefly in pernicious anemia, leukemia, hemolytic jaundice, etc., and to a less extent in secondary anemias. In considering poikilocytosis and anisocytosis due allowance must be made for the variation in size and shape that occurs, especially at the edge of slide preparations of normal blood. In the pathological condition the abnormality exists in all parts of the preparation.

Stippled erythrocytes suggest long standing toxic conditions, lead poisoning, primary anemia and malaria.

Polychromatophilia and nucleated erythrocytes indicate an attempt of the bone marrow to regenerate blood by a throwing out of immature or young cells, and are seen in the beginning of remissions in any of the severe anemias or during the recovery from blood loss due to any cause.

In many cases, especially of pernicious anemia, pathological erythrocytes appear in showers lasting for a few days, and then almost disappear for an equal or a longer period.

The absence of pathological erythrocytes in a case of anemia is evidence that the anemia is of mild degree. Secondary anemias of long duration occasionally tend to return to a normal color index, both the cell count and the hemoglobin per cent being reduced about the same.

Platelets. In addition to the leukcoytes and erythrocytes in the blood there are large numbers of platelets which vary greatly in size (generally 2 to 5 microns) and shape (Fig. 60) and are colorless. They stain only lightly, varying shades of purple and light blue, with Wright's stain (Plate II). The number, also, varies greatly in health, but still more in certain diseases. Very greatly increased or decreased numbers may be noted on the ordinary stained blood slide preparation.

The number of platelets can be determined sufficiently accurately for practical purposes by counting the number passed over on an ordinary blood smear (Fig. 27), while passing over (counting) a given number of leukocytes and then making the proper calculation

to determine the number of platelets per cubic millimeter. Count the platelets found while counting 25 leukocytes. Multiply this by 4 to get the number of platelets per 100 leukocytes. Multiply this number by the number of *hundreds* of leukocytes per cubic millimeter in the blood taken at the same time as determined by means of a total count. This gives the number of platelets per cubic millimeter. Count each separate platelet found in clumps so far as they can be made out. For instance, we would count 10 in Fig. 60.

Fig. 60. Photomicrograph of platelets stained with Wright's stain. Note variation in size and shape.

Interpretation of platelet counts. The normal count is usually between 200,000 and 500,000 per cubic millimeter. The number is greatly diminished especially in pernicious anemia and in purpura hemorrhagica, counts ranging from 50,000 or 60,000 downward to apparent total absence in some cases. The count is normal in hemophilia and increased in all septic or pyogenic diseases, chronic leukemia, trichinosis and following hemorrhage.

CHAPTER V

Blood—*continued*

BLOOD SUGAR DETERMINATION, FRAGILITY TEST, BLOOD MATCHING, BLEEDING TIME, COAGULATION TIME

Blood sugar determination (Myers and Bailey method)

Special apparatus required. (a) Colorimeter. The Duboscq type (Fig. 61) is recommended.

(b) Two test-tubes (regular 12 mm. by 115 mm. tubes) with 5 cc. mark.

Reagents required. (a) Picric acid, chemically pure. It is convenient to have several 0.25 gram portions, the amount required in the test, weighed out and wrapped in papers ready for use.

(b) Saturated solution sodium carbonate.

(c) Standard dextrose solution containing 0.2 gram pure dextrose in 1000 cc. saturated aqueous solution of chemically pure picric acid.

Method. 1. Draw about 2 cc. blood from vein and dilute with water before coagulation takes place; or "oxalate" by putting it in a tube in which a drop of a 20 per cent solution of potassium oxalate has been placed and allowed to dry. It is a good idea to have several of these oxalated tubes on hand ready for use.

2. Place 1 cc. blood in a small test-tube and add 4 cc. distilled water. Mix well and let stand until blood is completely laked.

3. Add 0.25 gram dry picric acid, stir well with a glass rod, and let stand for five minutes, stirring occasionally.

4. Centrifugalize to clear.

5. Transfer 1.5 cc. of the supernatant clear fluid to a tube marked at the 5 cc. level, and add 0.25 cc. saturated solution sodium carbonate.

6. Place 1.5 cc. of the standard dextrose picric acid solution in a similar tube and add 0.25 cc. saturated solution sodium carbonate.

7. Heat both tubes in a beaker or small pot or cup of boiling water for 15 minutes. Cool to room temperature.

8. Make up quantity in each tube to the 5 cc. mark with distilled water. Mix well.

9. Compare the unknown with the standard in the colorimeter, running the plunger down on the standard side to 20 millimeters and the one on the unknown side to where it matches the standard. Better take the average of two or three readings.

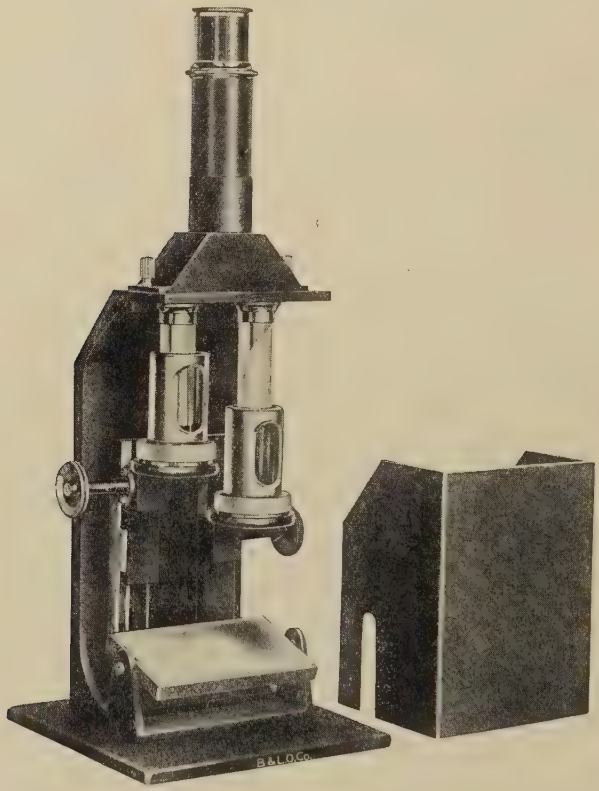


Fig. 61. Duboscq type colorimeter

Calculation.— $\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 100$ equals milligrams dextrose in 100 cc. of blood.

When blood sugar is very high a double strength standard must be used, in which case the result must be multiplied by 2.

Interpretation. Values below 80 mgm. constitute hypoglycemia; above 120 mgm., hyperglycemia. Interpretation of blood sugar findings is too complex to be presented here except to say increase to 150 to 250 mgm. is found in mild diabetes, while 300 to 400 mgm. or higher values are found in severe cases.

Erythrocyte fragility test

Draw 5 cc. of blood from median basilic vein (technic on page 167) with *dry* all-glass syringe.

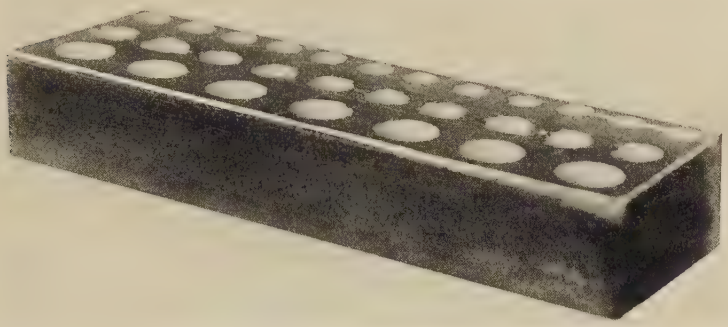


FIG. 62. Wood test-tube block. Holes may be bored of whatever sizes needed.

Place blood in clean, dry tube and defibrinate by slowly stirring with glass rod or wooden applicator for 5 minutes.

Arrange a row of 12 clean, dry tubes in tube block (fig. 62), and number these with grease pencil. The first tube starts with No. 25, the second No. 24, the third No. 23, and so on to the last, which is No. 14.

With 10 cc. graduated pipette filled with 0.5 per cent sodium chloride and held at angle of 45°, drop into each tube a number of drops corresponding to the label number.

Rinse pipette and fill with *distilled* water. Add to each tube the difference between its number and 25. (Thus the first tube is skipped, the second tube gets 1, the third tube 2 drops, etc.)

Shake all tubes well.

Add 1 small drop of defibrinated blood to each tube. Shake well and set aside for 1 to 2 hours. If the cells have not settled to the

bottom of tubes, centrifugalize a few seconds. Read the point at which hemolysis first begins to appear in the supernatant fluid, and the point where hemolysis is complete. The percentage of saline is found by multiplying the tube number by 2.

Interpretation. Hemolysis begins in normal bloods at 0.44 per cent saline and is complete at 0.34 per cent.

"Fragility" is *increased* if hemolysis occurs in stronger saline dilutions, and *decreased* if the cells are not hemolyzed in weaker solutions.

It is increased in pernicious anemia and decreased in hemolytic jaundice.

FIG. 63. Blood matching. Note agglutination of patient's cells by the donor's serum and absence of agglutination of donor's cells by patient's serum.

Blood matching

About 1 or 2 cc. of defibrinated (or citrated) blood from both patient and donor are required.

Place about 1 cc. of blood from each into test-tubes. Label "P" and "D." Centrifugalize until erythrocytes are well packed. Remove serum from each and transfer to separate tubes. Label "P.S." and "D.S." respectively.

Add about 9 volumes of normal saline to each tube of erythrocytes.

On a clean glass slide place one drop of serum from patient. Add 1 drop of cell suspension from donor. Spread out with a clean toothpick over an area about one inch square. Tilt the slide gently from side to side and look for evidences of macroscopic agglutination, which will occur, if at all, within a minute (Fig. 63).

Test the donor's serum against the patient's erythrocytes in same manner.

Interpretation. Agglutination in either case indicates incompatibility of the bloods, most important if the donor's cells are agglutinated by the patient's serum.

Bleeding time

Cleanse lobe of ear with alcohol, rubbing very *lightly*. Dry well. Make fairly deep puncture, which will allow the blood to flow without squeezing. With a blotter carefully remove the drops of blood as fast as they form. Note time required for bleeding to stop spontaneously.

Interpretation. Normal bleeding time, 3 to 5 minutes. Delayed bleeding time, 5 to 10 minutes. Prolonged bleeding time, 10 minutes or more.

Coagulation time

Prepare 5 or 6 capillary pipettes, having a lumen about the size of the lead in a pencil. These can be made by drawing out suitable glass tubing which has been heated in the flame of a Bunsen burner.

Squeeze out a large drop of blood from a rather deep ear puncture.

Fill the capillary end of the pipette by touching it to the drop and allowing the blood to run in by capillary attraction. Mark minute hand on watch.

On each half minute interval carefully mark with file and break the capillary portion containing blood and look for the presence of a fibrin thread between the fragments as they are slowly separated.

The appearance of the fibrin thread marks the time of complete coagulation.

Interpretation. Normal coagulation, 3 to 7 minutes. Delayed coagulation, 8 to 12 minutes. Prolonged coagulation, over 10 minutes.

CHAPTER VI

Malaria

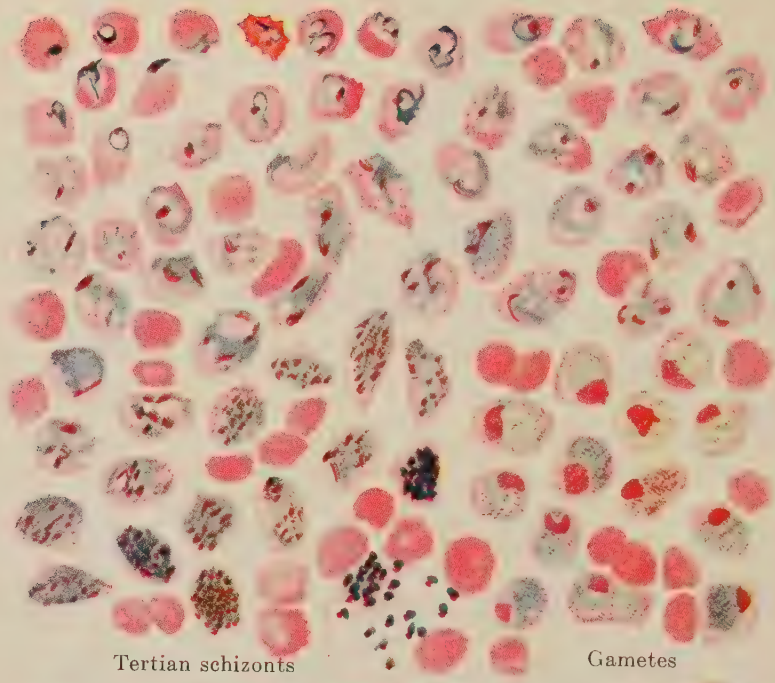
Obtaining blood and making preparations for examination for malaria plasmodia. There are more malaria plasmodia in the peripheral blood during the first six to eight hours following the onset of each paroxysm than at any other time, but there are sufficient at all times for a diagnosis to be made in practically all cases of malaria having active clinical symptoms at the time. The time to obtain specimens of blood for examination is therefore whenever malaria is suspected. Do not wait until any special time with reference to the occurrence of paroxysms.

Make an ordinary blood spread (see page 21). The specimen may be stained and examined at once or at your convenience, according to the demands of the case. Stain with Wright's stain.

Making the examination and recognition of plasmodia. Search for plasmodia only in parts of the preparation where the staining is good (nuclei of the leukocytes purple and the cytoplasm of the mononuclears a definite blue) and where the cells are not too thick. Prior to the acquisition of much experience with malaria plasmodia, never examine where the cells are more than one cell thick. After you have had sufficient experience you can recognize plasmodia satisfactorily where the spread is two or three cells thick and of course at a considerable saving of time, since the thicker the blood film the more plasmodia are present in a given area.

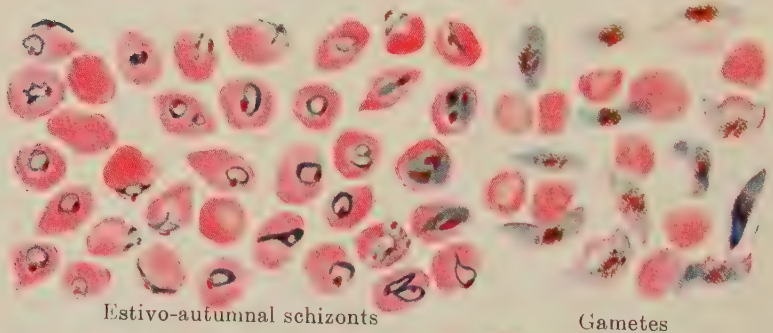
Malaria plasmodia are in (or on) the erythrocytes, and no object should be considered as probably a plasmodium unless it is so situated. It is true that many plasmodia, especially old gametes, are in (or on) little more than "shadows" of erythrocytes, the cells having faded to a great extent.

The cytoplasm stains blue, the chromatin stains the usual red, like chromatin in the nuclei of cells, platelets, etc. Pigment granules when present do not stain but have a light to dark brown color. Do not expect to find malaria plasmodia well stained in preparations where the leukocytes and platelets are not well stained. Unless



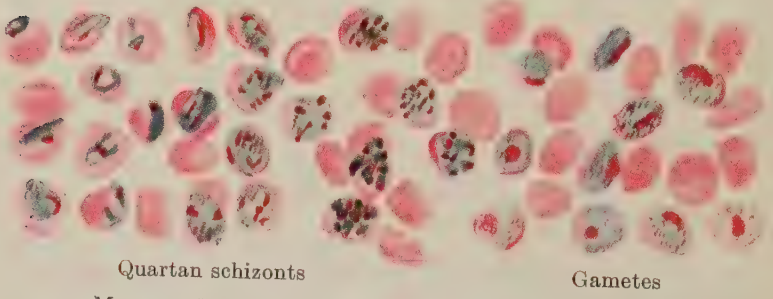
Tertian schizonts

Gametes



Estivo-autumnal schizonts

Gametes



Quartan schizonts

Gametes

MALARIA PLASMODIA FOUND IN PERIPHERAL CIRCULATION

the nuclei of leukocytes have the proper reddish tint due to proper staining of the chromatin in them, the chromatin of the plasmodia will not be properly stained. In examining a blood specimen for malaria plasmodia, one should make a habit of noting, upon first looking at the preparation, whether the staining is good, and not look for plasmodia in any but well stained preparations and good areas of such preparations. The staining often varies greatly in different areas on the same slide. Perfectly satisfactory areas may be found on slides on which there are other very unsatisfactory areas. Looking over the preparation with the low power lens greatly facilitates the selection of suitable, well stained areas for examination.

The number of plasmodia present varies very much. Often there may be several plasmodia in every field, while in other cases there may be so few that a search of several minutes may be required before a single plasmodium is found.

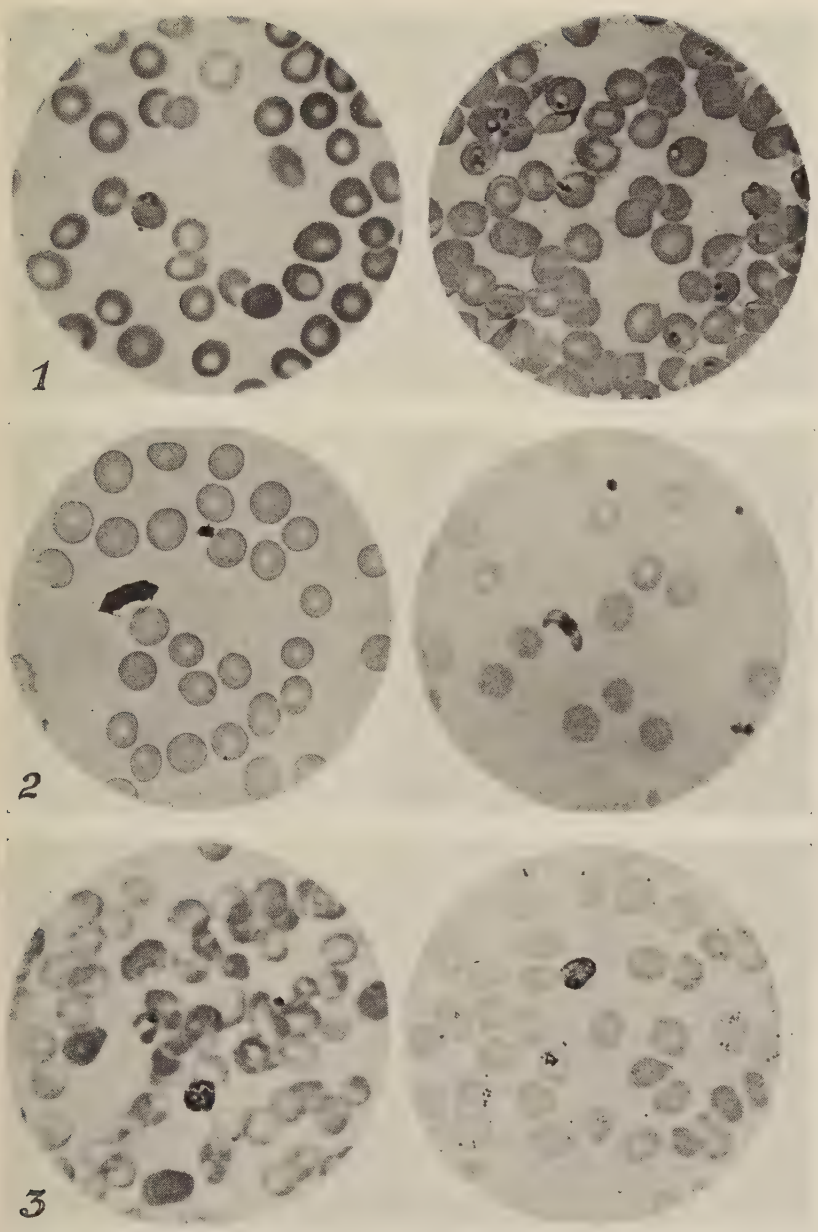
Description of malaria plasmodia. There are three distinct species of malaria plasmodia now known to infect man, and it is highly probable that it may be found that still others exist. They are (1) estivo-autumnal, *Plasmodium falciparum;* (2) tertian, *Plasmodium vivax* and (3) quartan, *Plasmodium malariae* (Plate VI). They all grow and reproduce in the blood of man in the same general way. The smallest plasmodium consists of a thin round wall of basophilic cytoplasm enclosing a round or oval mass that does not stain. This gives the appearance of a ring. There is one, and sometimes there are two granules of red-stained chromatin at one side of the ring, giving rise to the designation "signet ring." As the plasmodium grows the erythrocyte is slowly consumed and after about twelve to twenty-four hours there appear light to dark brown granules of pigment. These become more numerous until the development of the parasite is complete. An individual plasmodium reaches maturity in about forty-eight hours in the case of estivo-autumnal and tertian, and in seventy-two hours in the case of the quartan parasite. The chromatin granule grows in mass for about half the developmental period, after which time it begins to divide by segmentation. Division goes on slowly until at the end of the period the number of segments, peculiar to the particular species, have been formed. Toward the end of the period, division of the cytoplasm of the organism occurs, and now the whole plasmodium is made up of a number of

separate chromatin granules each having its own separate portion of cytoplasm. Such a mature, or segmented plasmodium is called a "rosette." Finally the capsule of the rosette ruptures and each segment or young plasmodium, known as a *merozoite,* is capable, under favorable conditions, of attaching itself to an erythrocyte and passing through a similar cycle of development, giving rise in turn to another crop of merozoites. This kind of reproduction takes place without sexual influence and is known as *schizogony.* Asexual plasmodia (of any age) are schizonts.

After a person has been infected with malaria plasmodia for more than two or three weeks, and as a result of influences not now well understood, certain plasmodia, instead of developing through the asexual cycle of schizogony just described, become sexually differentiated and take somewhat different form. These are supposed not to be capable of reproducing in man. The males are capable of giving rise (in the mosquito) to microgametes, and the females to macrogametes. They are capable of reproducing in the mosquito by what is called *sporogony.* Sexual plasmodia in the blood are correctly called gametocytes, but in common usage the term gamete is used. Estivo-autumnal gametes are crescent shaped, but tertian and quartan gametes (plates VI, VII and VIII) are round or oval and resemble very much the developing, almost mature schizonts. The chromatin in gametes in the blood does not divide as it does in the schizonts.

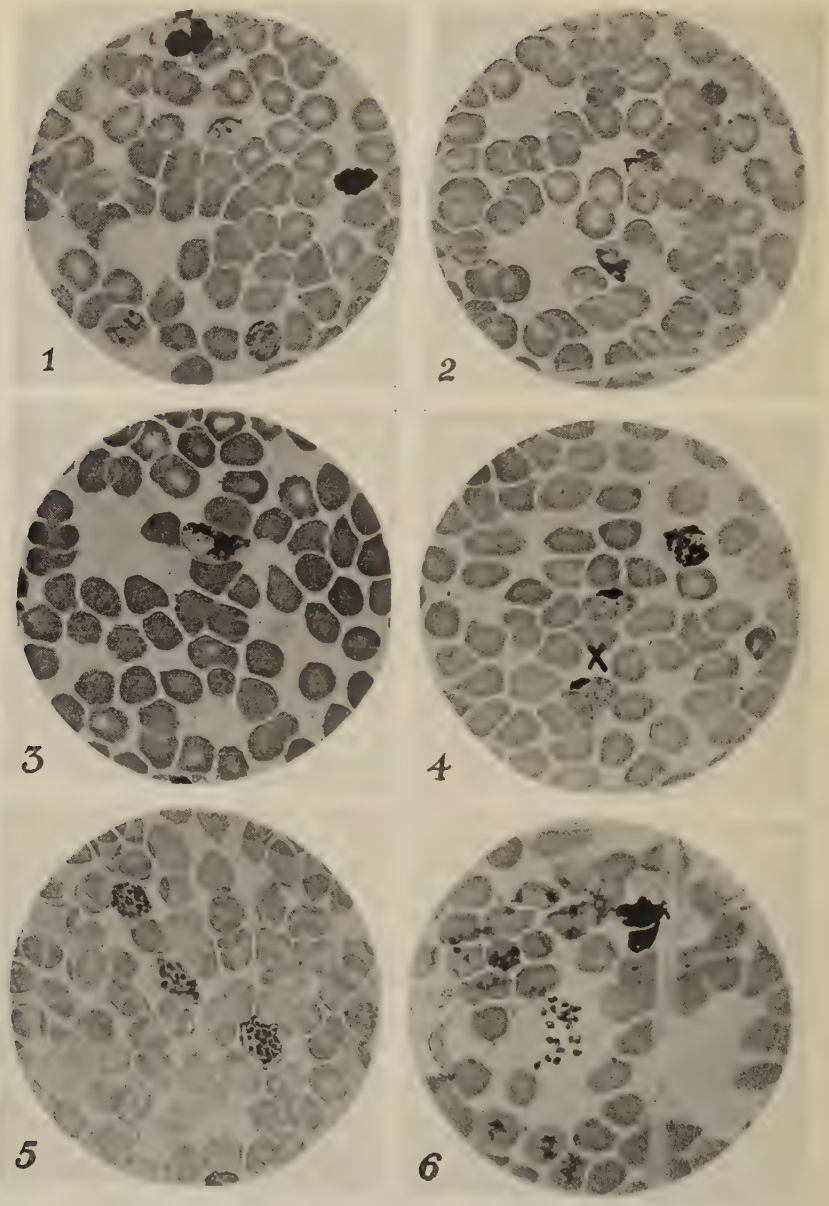
Estivo-autumnal schizonts disappear from the peripheral circulation and lodge in the capillaries when they are about twelve hours old. They do not reappear in the peripheral blood until after segmentation (except extremely rarely). For this reason practically the only estivo-autumnal schizonts found in peripheral blood are the ring forms and young parasites.

The tertian and quartan schizonts begin to disappear from the peripheral circulation after about two-thirds of their period of development has passed, but on account of the fact that they are capable of active ameboid movement, they often pass out of the capillaries in which they lodge and reappear in the peripheral circulation. This fact that all ages of tertian and quartan schizonts are present in peripheral blood and only the ring stage of estivo-autumnal schizonts constitutes a most valuable means of differentiation between different species.



PHOTOMICROGRAPHS OF SCHIZONTS AND GAMETES, ESTIVO-AUTUMNAL AND QUARTON PARASITES

1. Estivo-autumnal parasites—schizonts
2. Estivo-autumnal parasites—gametes.
3. Quartan malaria. Schizont (left). Gamete (right).



PHOTOMICROGRAPHS OF TERTIAN SCHIZONTS, GAMETES AND MEROZOITES

Tertian malaria. Cycle of schizogony.

1. Post-ring schizonts.
2. Quarter grown.
3. Half grown (nuclear division).
4. Full grown. Note gametes, *X*.
5. Segmenters.
6. Merozoites.

The gametes of all species appear in the peripheral blood. However, not all cases have gametes in their blood all the time. Schizonts may be present and no gametes or vice versa. Gametes are found in from 10 to 50 per cent of the general run of cases of malaria.

Differentiation of malaria plasmodia. The rings of different species look very much alike and it is not practical to diagnose species by the rings. The very small rings found in pernicious estivo-autumnal malaria may be differentiated from those of the so-called benign estivo-autumnal or tertian and quartan parasites. They are extremely small and are often overlooked. The presence of other size parasites than rings shows either tertian or quartan infection. The absence of them indicates (but does not absolutely prove) that the rings are estivo-autumnal.

Infection with more than one species occurs, but is rare. The most useful differential points are shown in the following table.

	ESTIVO-AUTUMNAL	TERTIAN	QUARTAN
Schizonts found in peripheral blood.	Rings only.	All sizes.	All sizes.
Shape of gametes.	Crescentic and oval.	Approximately round.	Approximately round.
Shape of schizonts found in peripheral blood.	Rings. May have two chromatin granules.	Very irregular after ring stage passed.	Regular after ring stage is passed. Some band forms.
Influence upon color of erythrocyte.	Not changed.	Faded, pale.	Darker. Normal red color intensified.
Influence upon size of erythrocyte.	Not changed.	Enlarged.	Reduced.
Pigment granules.	Medium to coarse.	Very fine.	Coarse.
Schüffner's granules.*	(Not present)	Often present.	Not present.
Number of segments in rosette.	(16 to 32)	24 to 32.	6 to 8.
Period of asexual development.	(48 hours)	48 hours.	72 hours.

* Red staining granules in the erythrocyte containing the parasite.

Statements included in parentheses are not usually determinable in ordinary blood spreads.

Interpretation of examination of the blood for malaria. There are always sufficient plasmodia in the peripheral blood to enable a competent examiner to find them in a search of ten minutes or less in the case of all persons who have active malarial fever. It is possible in practically all cases, therefore, to determine positively that an existing fever, or chills and fever, is (or is not) due to malaria.

If quinine has been taken by the patient during the 48 hours previous to the time the blood is taken for examination, this may cause great reduction or complete disappearance of plasmodia from the blood. Therefore, in interpreting negative findings, the question as to whether quinine has been taken recently should always be raised. However, it should be remembered that quinine causes clinical symptoms of malaria to disappear *more quickly* than it does the parasites.

It is often possible to find plasmodia in the blood of people who have chronic or latent malaria by thorough and repeated examinations, but it is not possible to examine the blood and to determine that the patient has not some plasmodia in some of his organs and tissues. If he had enough to make him sick, however, they could be found readily. Therefore, negative findings do not eliminate the possibility of latent malaria infection. They do, however, practically eliminate malaria as a cause of any active clinical symptoms present at the given time.

The number of plasmodia present in the blood does not always coincide with the clinical symptoms. Sometimes parasites are quite numerous when symptoms are mild and vice versa.

CHAPTER VII

Typhoid Agglutination Test

This is a test to determine the presence or absence in the blood of a specific substance, typhoid agglutinin, which has the property of causing typhoid bacilli to agglutinate or collect together in clumps. Typhoid agglutinin will agglutinate either living or dead typhoid bacilli. Many different methods of making the typhoid agglutination test are employed in different laboratories. The macroscopic slide test known as the Bass-Watkins test has the advantage of not requiring any special laboratory facilities, or expert technical skill, being easily made by the physician himself at the bedside if desirable, and requiring only two or three minutes for its completion. When the test is made with reliable material and with reasonable accuracy, it is sufficiently reliable and accurate for practical clinical needs. It is possible to make more accurate dilutions of serum in the microscopic test, but a living culture of typhoid bacilli is required and also laboratory facilities and technical skill necessary for such work. In order to meet the demands of those who have such facilities and skill and who prefer to make the microscopic test, we describe a convenient method of making it, in addition to the method of making the macroscopic slide test, which will probably be most frequently used, except perhaps in large, well equipped laboratories.

The macroscopic test. The special material required is a suspension in distilled water of typhoid bacilli, ten billion per cubic centimeter, killed and preserved with 1 per cent commercial formalin. At present this can be obtained from the Laboratory of Clinical Medicine, Tulane University, School of Medicine, New Orleans, La., at $1.00, in advance, for a 2-drachm bottle. It keeps well for at least several months if kept tightly corked. Shake before using. When a vial of it is frequently opened and used from, it should be discarded after a few weeks and a new supply should be obtained. An ordinary medicine dropper and a wood toothpick are also needed.

Make the regular blood-spread on a slide, using approximately one-fourth drop of blood. The test may be made at once at the bedside,

or it may be made at any time at your convenience, within a week or two. Place on the blood one drop of water. Carefully spread this over the film of blood (Fig. 64) with the end of a pick or other suitable instrument. Tilt the slide from side to side to hasten the

FIG. 64. Spreading the drop of water over the blood to dissolve it in making typhoid agglutination test. Do not allow the pick to *scrape* the slide.

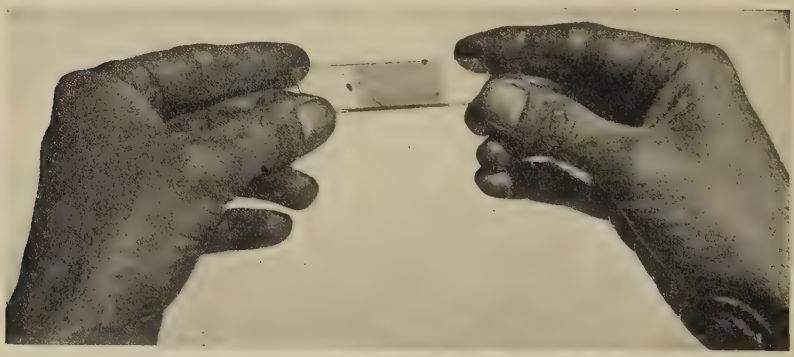


FIG. 65. Tilting slide back and forth to facilitate mixing and to hasten agglutination.

dissolving of the blood. A clear solution of approximately one-fourth drop of blood in one drop of water is thus made. Now add one drop of the suspension of typhoid bacilli and mix by tilting the slide from side to side (Fig. 65) and from end to end, causing the mixture to flow back and forth. This agitation hastens the reaction. If the

blood contains the specific agglutinin for typhoid bacilli, the millions of bacilli present soon begin to collect together, first in small grayish clumps (Fig. 66) appearing as a fine granular sediment. Later the clumps of bacilli become coarser and are readily recognized (Figs. 67 and 68). The reaction takes place and is complete within two minutes. When the test is negative no such granular sediment forms.

Practise with known typhoid and known negative blood until you are familiar with the test. Make many such tests until familiar

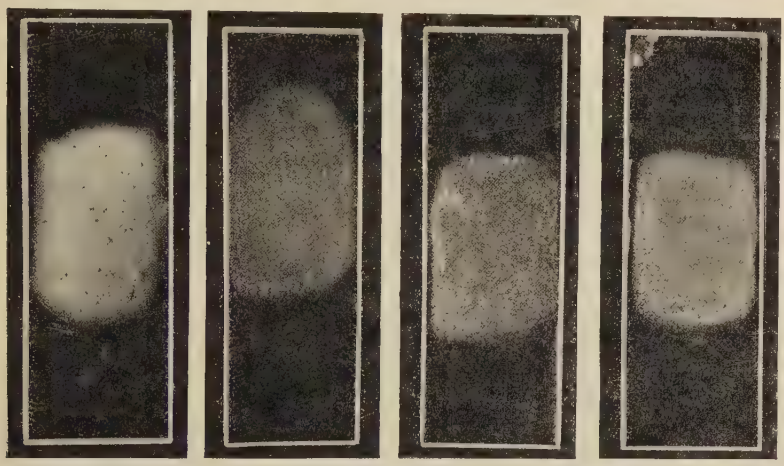


FIG. 66. A series of agglutination tests. (a) Negative test. (b) Weak positive reaction. (c) Moderate positive reaction. (d) Strong positive reaction.

with the appearance of positive and negative reactions. Do not mistake dust particles for agglutinated bacilli. Doubtful reactions occur in this test just as in all other tests for specific antibodies which vary in amount from none to sufficient to produce definite positive reactions.

The microscopic test. A reliable pure culture of typhoid bacilli is required. It is most convenient to carry the stock culture on standard agar, transplanting every week or ten days. Growth is usually satisfactory at room temperature in most instances and a special bacteriological incubator is not necessary, provided care is taken to keep the cultures in a warm part of the room.

When a test is to be made, a fresh, 24-hour-old culture is required. Inoculate a tube of nutrient broth and allow it to grow for twenty-four hours. If broth tubes are not available, satisfactory results can

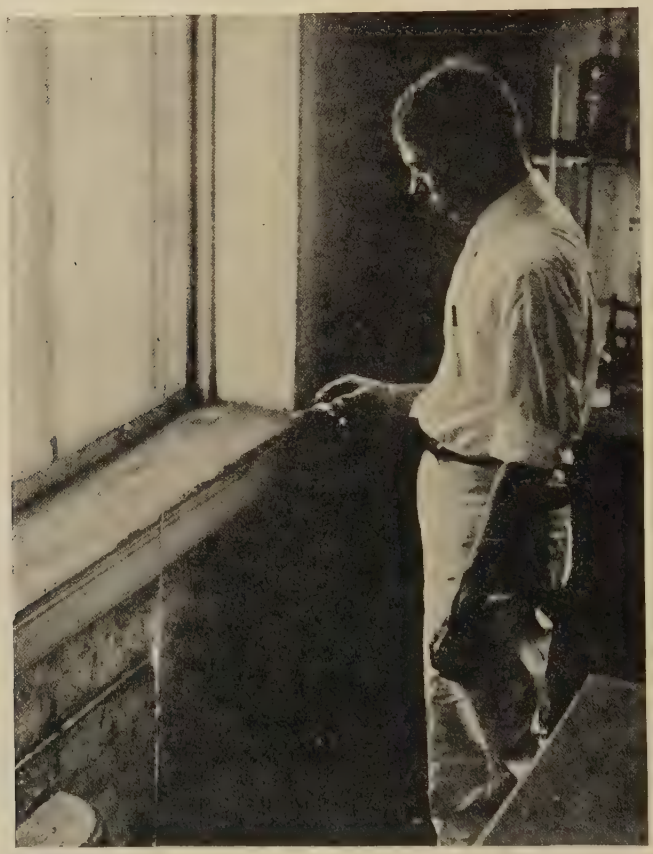


FIG. 67. Looking at slide in typhoid agglutination test. The granules can be seen best when there is a dark background in the distance and the light falls upon the specimen from the side.

be obtained by washing off the 24-hour growth on an agar slant with 5 to 10 cc. of water. Either this suspension of typhoid bacilli or the broth culture is to be used for the microscopic test. The object is to mix equal parts of the culture and the serum to be tested, and ob-

serve under the microscope whether the bacilli are agglutinated or not.

Take an ordinary blood spread (see page 21), which is made of about one-fourth drop of blood and therefore contains about one-eighth drop of serum. Dissolve this with three drops of water, making a dilution of serum about 1 to 25. If the amount of blood on

FIG. 68. Looking at typhoid agglutination test, at night by the light of a match held in the right hand beneath and to the side of the specimen.

the slide is estimated to be more or less than the usual one-fourth drop, use more or less water accordingly. If pure serum, separated from the clot, is to be tested instead of the blood spread, make a 1 to 25 dilution in any convenient way and test in the same manner.

Place on a slide, near the middle, three loops of the diluted blood or serum and three loops of the broth culture or suspension from the agar culture (Fig. 69). Each loopful is placed near but not touch-

ing the others. Now mix by stirring all together with the platinum loop. Put on a cover glass. It is a good idea to place a cotton fiber or two in the mixture to keep the cover glass from mashing it too thin. Examine the preparation under the microscope—"high dry" objective—and note that the bacilli are actively motile and there is no clumping. Now put the preparation aside and examine again in 15 to 20 minutes. If the test is negative, the bacilli will still be motile. If it is positive, the bacilli will have ceased moving and mostly

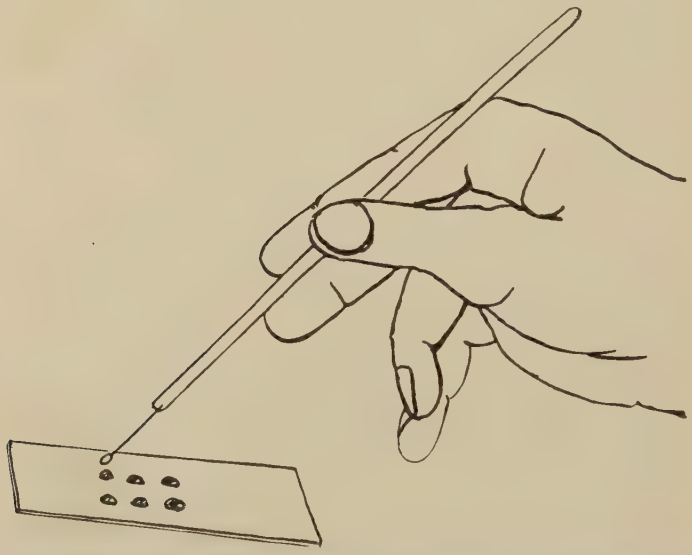


FIG. 69. Making preparation on slide for microscopic typhoid agglutination test. Three loops (small droplets) of diluted blood and three of typhoid culture have been placed on the slide near to but not touching each other. They are then mixed by stirring with the platinum loop.

collected together in small clumps of varying size, (Fig. 70) each containing many bacilli.

Interpretation of the typhoid agglutination test. About 10 to 20 per cent of all cases of typhoid give positive reactions by the end of the first week. About 70 per cent give positive reactions by the end of the second week. More than 90 per cent give positive reactions at some time during the course of the disease. A few do not give positive reactions at any time. Usually the reaction may be obtained

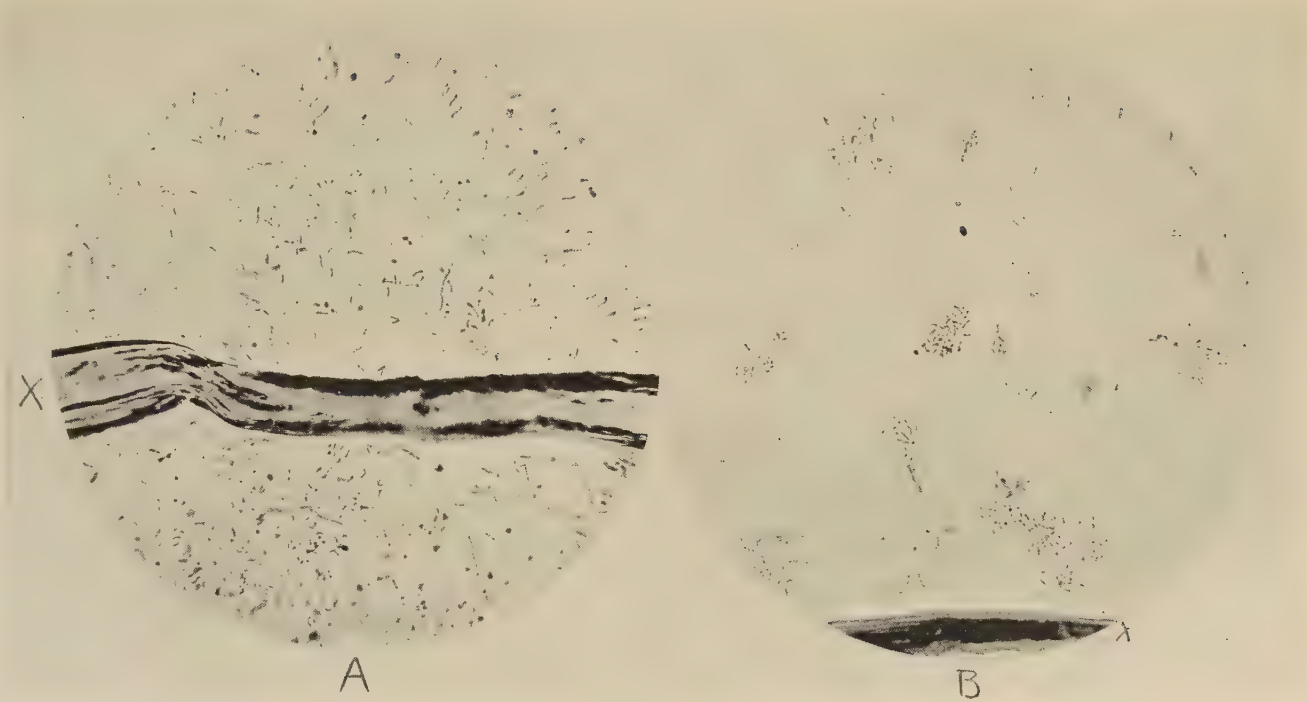


FIG. 70. Indicating microscopic agglutination; (A) bacilli not agglutinated—negative. (B) bacilli agglutinated—positive.

until after the fever subsides. The reaction gradually gets weaker as time passes following convalescence, and after three to six months it is weak or negative in the majority of cases. A few give positive reactions for a year or two and sometimes longer.

A positive reaction, if accompanied by clinical evidence, is practically diagnostic of typhoid. A negative reaction does not prove that the patient has not typhoid. Its negative value is in proportion to the duration of the disease. Given a case of fever (possibly typhoid) of two weeks' duration, for instance, the indication of a negative reaction would be 70 per cent that it is not typhoid. Here, as in most other laboratory findings, the test is of most value when interpreted in connection with the clinical evidence.

Blood from persons who have been vaccinated against typhoid usually gives a positive agglutination test for some days or weeks thereafter. In attempting to attach the proper diagnostic significance to a positive test, due consideration should be given to previous vaccination.

The same test and technic may be used in the diagnosis of paratyphoid fever provided one uses similar suspensions or cultures of paratyphoid bacillus A, and paratyphoid bacillus B, in place of the suspension of typhoid bacilli.

CHAPTER VIII

Urine

Collection of specimens for examination. The only proper specimen of urine for examination is a recently voided one, except when it may be desirable to make a quantitative examination of the total urine voided during twenty-four hours. No method of preservation will keep specimens in perfect condition for all examinations. Different specimens keep variable lengths of time. Some are not good after an hour or two, while others are satisfactory for at least ten or twelve hours, and sometimes longer. As specimens get a few hours old, contaminating bacteria, which often multiply very rapidly, alter the formed elements, such as blood cells, casts, etc., that may be present, cause crystallization and precipitation of the salts and affect, by fermentation or otherwise, such substances as sugar and albumin that may be present. Sometimes the amorphous or crystalline sediment that forms in a short time is so great that it renders microscopic examination impractical or at least unreliable. Do not examine old specimens unless as a last resort.

Patients who come to the laboratory or office should void urine there for examination, but if inconvenient they may be instructed to void just before leaving home to come to the office and to bring a sample of the fresh urine only. Specimens to be sent to the laboratory should be voided just before they are sent. Examination of the early morning urine is sometimes the most valuable, especially with regard to ascertaining the ability of the kidneys to excrete a urine of normal specific gravity. Instruct the patient to void in a *clean* vessel or urinal and to send about four ounces in a *clean* bottle. Larger quantities are unnecessary for routine examinations.

If significance is to be attached to the presence of pus, instruct the patient to void if possible into two clean glasses and to send two to four ounces from the second glass in a clean bottle for examination. Females should be instructed further to thoroughly wash the vulva with soap and water before voiding. Some physicians take this precaution in all cases as routine. Catheterized specimens are the

only kind from females that can be depended upon as certain not to contain pus and acid-fast bacilli from the vulva. We do not advise catheterization for routine purposes. It is not necessary and may result in infection.

Physical examination

Specific gravity. A urinometer (Fig. 71) is required. One known as Squibb's urinometer is sufficiently accurate for ordinary purposes.

Fig. 71. Urinometer. Testing specific gravity of urine. Note curve of meniscus.

Fill the tube nearly full of urine. Put in the float. Hold level with the eye and read at the bottom of the meniscus. Take care that the float does not touch the sides of the tube.

Chemical examination

Test of the reaction. Dip a strip of blue and a strip of red litmus paper in the urine. If acid, the blue paper changes to red. If alkaline, the red paper changes to blue.

Test for albumin. (A) Qualitative. Fill a test-tube three-fourths

FIG. 72. Boiling urine in test for albumin

FIG. 73. Adding acetic acid to urine from a drop bottle in testing for albumin

full of *cleared* urine. Very cloudy urine should be cleared before it is tested. This is best done by centrifugalization. The supernatant urine obtained by centrifugalizing for microscopic examination is especially suitable for this and other chemical tests. By pouring it off into another tube we have in one tube clear urine for chemical tests and the sediment for microscopical examination in another. Hold by the lower end in the hand. Tube holders are unnecessary. Boil (Fig. 72), for a minute or two, approximately the upper one inch

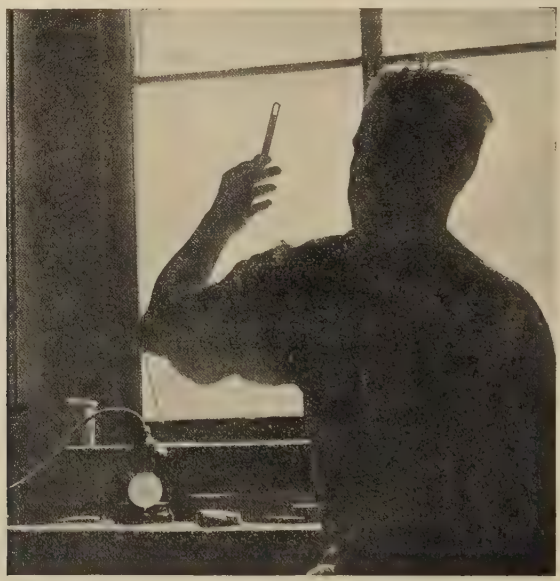


FIG. 74. Looking for faint cloud in test for albumin. Dark background in the distance with light falling on the tube from the side.

of the column by holding in the flame of a small Bunsen burner or alcohol lamp. Revolve and agitate while boiling to prevent boiling over. If albumin is present, a cloud (Plate IX), varying in intensity with the amount present, will appear. Phosphates also produce a cloud upon boiling. Now add a few (4 or 5) drops of glacial acetic acid (Fig. 73). If the cloud is due to albumin, it will be intensified. If it is due to phosphates, it will be cleared up. A faint cloud due to only a trace of albumin can be seen best by holding the tube (Fig. 74)

FIG. 75. Cloud due to albumin in urine, as seen in the proper light against a dark background.

FIG. 76. Preparing fresh Fehling's solution in tube. Tube contains the copper solution. Adding equal amount of alkaline solution, using little finger to indicate level to which this is to be added.

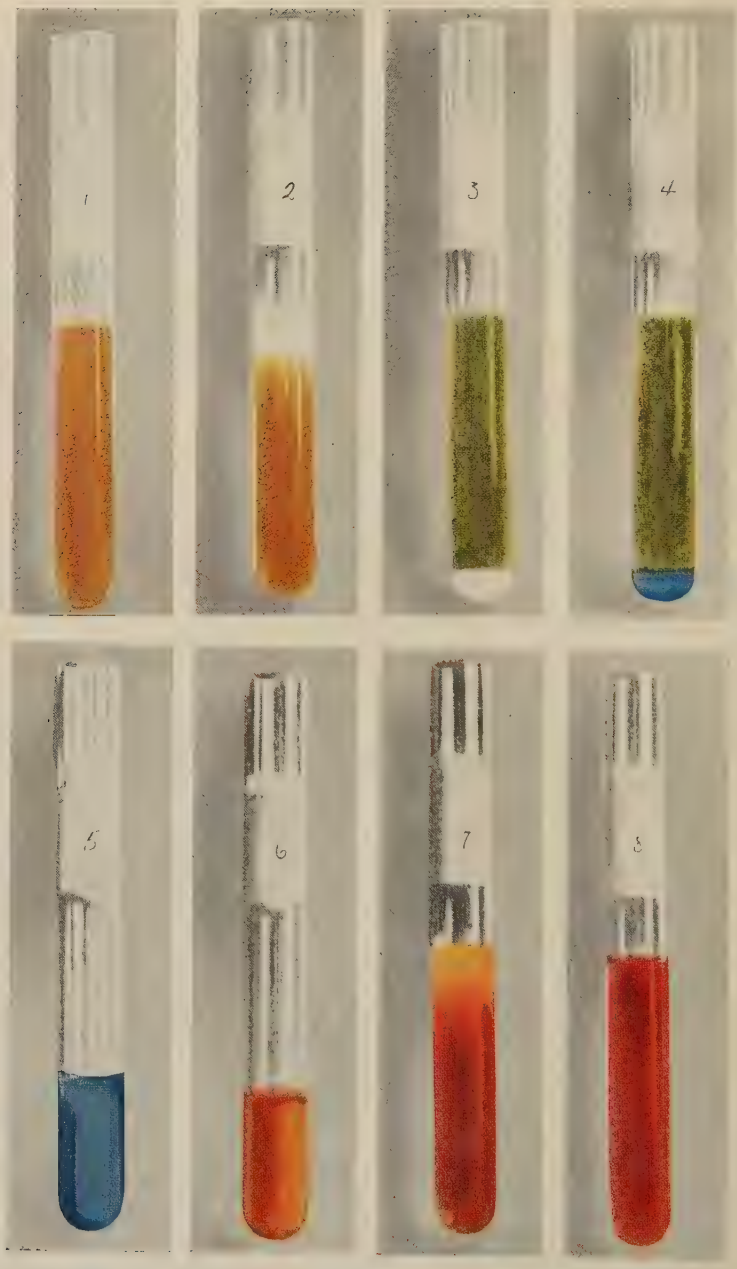
so that it has a dark background a few feet away and has light (from window or otherwise) falling upon it from the side (Fig. 75).

(B) Quantitative. There is no practical quantitative test for albumin that is absolutely accurate. The one here given is sufficiently accurate for practical purposes. Fill a graduated Purdy tube with clear urine to the 10 cc. mark. Add 2 cc. of glacial acetic acid and 3 cc. of a 10 per cent solution of potassium ferrocyanide. Shake and allow to stand a few (2 to 5) minutes. Centrifugalize until the coagulated albumin has packed well in the bottom of the tube (about 2 or 3 minutes). Read off the volume of precipitate on the graduations on

Fig. 77. Boiling Fehling's solution and urine in testing for sugar

the tube, each 0.1 cc. mark representing 1 per cent. When the amount of albumin is excessive, dilute the urine until the volume of precipitate falls below 1.5 cc. and multiply the result by the dilution. To get the percentage by weight of dry albumin, multiply the amount of sediment in cubic centimeters by 0.21.

Test for sugar. Qualitative. There are several tests to select from. We give Fehling's test here because it is generally required by life insurance companies, for whom no doubt many of our readers will be called upon to make examinations. We require two separate stock solutions, Fehling's alkaline solution and Fehling's copper sulphate solution (Formula 4).



CHEMICAL TESTS OF URINE

(See page 89 for Explanation of Tubes)

These two separate solutions keep well, but when mixed together the resulting "test" solution does not keep more than a few days. It is therefore desirable to prepare the test solution at the time a test is made by mixing together equal parts of the alkaline solution and the copper solution. Keep the separate solutions in glass stoppered drop-bottles. The drop-bottles are more convenient to pour the solutions from.

To make a test, drop into a test-tube a few drops, not more than one-half cc., of one solution, hold the finger as a marker (Fig. 76) at the place to which the solution will rise when approximately an equal volume of the other solution is added. Add the other solution, shake, and we now have about 1 cc. of a mixture of approximately equal parts of the two different solutions, which constitutes Fehling's test solution. Heat the solution to boiling over a Bunsen burner (Fig. 77) or alcohol lamp, then add about one-half as much urine and boil again. If sugar is present, the solution assumes an opaque yellow color (Plate IX) and shortly after a dense yellowish-red sediment falls to the bottom. Shake the tube while heating to prevent boiling or "popping" out or put in two or three small pebbles. No tube-holder is necessary.

Benedict's qualitative test for sugar is considerably more sensitive than Fehling's test and has certain other advantages. It is probably the most popular and widely used test. Its great sensitivity has been objected to by some. Traces of sugar appearing in the urine of normal individuals from the slightest provocation and shown by this

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Explanation of Plate IX

Tube 1. Test for albumin. No cloud after boiling and addition of acetic acid.

Tube 2. Test for albumin. Cloud produced by boiling and remaining after addition of acetic acid, thus showing presence of albumin.

Tube 3. Test for indican. Negative reaction. Chloroform not colored.

Tube 4. Test for indican. Positive reaction. Chloroform colored blue.

Tube 5. Test for sugar with Fehling's solution. Negative reaction. No change in color.

Tube 6. Test for sugar with Fehling's solution. Positive reaction. Opaque yellowish red color due to reduced copper.

Tube 7. Test for acetone. Negative reaction. Disappearance of red color upon addition of acetic acid.

Tube 8. Test for acetone. Positive reaction. No disappearance of red color upon the addition of acetic acid.

test are likely to be misconstrued as pathological. With good judgment in making interpretations, this great sensitivity is not an objection but rather an asset.

To about 1 cc. of Benedict's qualitative reagent in a test-tube add two or three drops of urine. Boil for a minute or two. To prevent bumping and popping out, shake the tube lively as it is being boiled. Better still, put in two or three small pebbles. A supply of these should be on hand for use when boiling anything in test tubes. No tube holder is necessary.

If sugar is present, a general turbidity—green, yellow or red—the color depending upon the amount of sugar present, develops. A clear solution or a whitish turbidity, due to precipitated urates, is negative.

(B) Quantitative test for sugar. There are several different quantitative tests for sugar. Benedict's test seems to meet with most favor. The method given here of making the Benedict test is sufficiently accurate for ordinary purposes, is convenient and requires the minimum of apparatus and material.

Measure 1 cc. of Benedict's quantitative solution (Formula 6), into a test-tube by means of 1 cc. graduated pipette. Put in a pinch of (about 0.25 gram) sodium carbonate and two or three small pebbles. With another pipette (or with the same one after washing), graduated in hundredths, take up some of the urine to be tested and after noting the starting point, add a few hundredths of a cubic centimeter to the solution at a time (Fig. 78), boiling a little between each addition. Touch the tip of the pipette to the side of the tube so that all urine let out will flow into the tube. A chalk-white precipitate forms and the blue color begins to fade. Add a little more urine slowly, a hundredth or two at a time, until the last trace of blue disappears.

Now read off on the pipette the amount of urine used and calculate the per cent of sugar indicated by the test. One cubic centimeter of Benedict's solution requires 0.002 gram of glucose to remove the blue color, and therefore the amount of urine used contains 0.002 gram of glucose. To calculate the per cent of glucose present by a short rule, divide 0.2 by the amount of urine used expressed in cubic centimeters. The quotient is the per cent of glucose present. For example, if 0.20 cc. of urine is required, $0.2 \div 0.20 = 1$, the per cent of sugar indicated. If a large amount of glucose is present (3 per

cent or more), it is better to dilute the urine, say ten times, with water and to test this diluted urine. Of course, the amount of sugar present in the diluted urine must be multiplied by the number of times the urine was diluted, to determine the amount in the undiluted urine.

FIG. 78. Adding urine in 1 cc. graduated pipette to Benedict's solution in quantitative test for sugar. Note tip of pipette touching inner side of tube.

Test for indican. Place about 2 cc. of urine in a test-tube. Add a few drops (6 or 8) of Fehling's copper solution, about $\frac{1}{2}$ cc. of chloroform and then approximately an equal quantity of hydrochloric acid. Close the mouth of the tube with the thumb, shake thoroughly and then allow the chloroform to settle to the bottom. If indican is present the chloroform assumes a blue color (Plate IX), varying in

intensity with the amount of indican present, but it remains uncolored if indican is not present. A red color indicates the presence of iodides.

Test for acetone. To about 3 cc. of urine add several drops of a deep red solution of sodium nitroprusside. Add several drops of Fehling's alkaline solution. If acetone is present the solution should turn a deep red color which is intensified by the addition of acetic acid. If acetone is not present the addition of acetic acid immediately decolorizes the acidified portion of the tube contents. (Plate IX.)

Test for di-acetic acid. To about 3 cc. of urine in a test-tube add a few drops of 10 per cent solution ferric chloride, drop by drop. Centrifugalize the tube to clear the solution, and add another drop or two of ferric chloride. If di-acetic acid is present a deep purple or port wine color is produced. This same reaction is given when the urine contains elimination products of coal tar derivatives.

Test for bile. Stopper the specimen bottle well and shake vigorously. A greenish discoloration of the foam indicates the presence of bile.

A slightly more sensitive test is made by overlaying urine with a lemon-yellow watery solution of iodine, Lugol's solution or tincture of iodine, whereupon a green ring develops at the junction of the two solutions. This ring is best seen by holding the tube against a piece of white paper.

Test for urobilin. To 2 cc. of urine in a test tube add several (3 or 4) drops of Lugol's solution, and an equal quantity of a saturated alcoholic solution of zinc chloride. Centrifugalize to throw down the precipitate. Examine the tube while holding it in *direct* sunlight or in the rays from a beam of strong light. A greenish fluorescence indicates the presence of urobilin.

Phenolsulphonephthalein kidney function test. Have the patient empty the bladder and drink a large glass of water. Take up into an all-glass syringe, that has just previously been sterilized by boiling, the contents of an ampoule of phenolsulphonephthalein solution (made by Hynson, Westcott, and Dunning). Blow out any air bubbles and adjust to the 1 cc. mark. Have patient swell up vein at the bend of the elbow by grasping the arm above to constrict

it and closing tightly the hand of the same arm. Apply tincture of iodine to spot selected for puncture. Insert needle into vein. As soon as blood starts flowing back into syringe, have constriction relaxed and inject exactly 1 cc. of the dye solution. It may be given intramuscularly instead of intravenously, but if so, allow an extra ten minutes for absorption.

Collect all urine voided during the first hour following injection, having patient void at end of first hour. Collect all urine voided during the second hour. Test each specimen separately.

FIG. 79. Dunning's colorimeter (or "comparometer"). Convenient in making phenolsulphonephthalein kidney function test. The Duboscq colorimeter (Fig. 61) has a wider range of usefulness but is more expensive.

Pour urine in 1000 cc. graduate. Add about 5 cc. of 5 per cent solution sodium hydroxide. Dilute with water to the 500 cc. mark. If red color is intense, dilute to the 1000 cc. mark. Mix by covering graduate with the palm of the hand and inverting. Now read percentage of dye present by comparison with known standard in a colorimeter, of which there are many different types available. Dunnings' colorimeter (or comparometer) (Fig. 79) is entirely satisfactory for this purpose. Fill colorimeter cup, match it with the standards and note the percentage indicated. If dilution was only 500, divide reading by 2. If another make of colorimeter is used, a standard

solution for comparison can be made by adding some of the dye-solution to alkalinized water; 0.1 cc. of dye from ampoule to 100 cc. of water gives a 100 per cent standard solution.

Urea concentration test. This is a test of the ability of the kidneys to concentrate urea, the most important end-product of exogenous nitrogenous katabolism representing about 85 per cent of the total amount of nitrogen eliminated through the kidneys.

Patient should abstain from food or drink for at least two or three hours before the test is started. He voids urine and then takes 15 grams urea dissolved in 100 cc. of water, flavored, if desired, with tinctura aurantii. Patient voids at end of first hour and of second

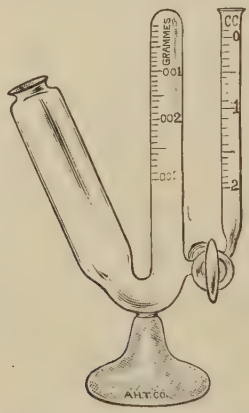


FIG. 80. Doremus-Hinds ureometer.

hour. Specimens are kept and tested separately. Test the second specimen first as it is generally the highest. If it shows a value of 2 per cent (some consider 2.5 per cent as the low normal limit) or above there is no need to test specimen No. 1.

To determine the per cent of urea, the hypobromite method with the Doremus-Hinds ureometer is convenient and practical. Pour some of the urine into the smaller tube of the apparatus and then open the stopcock and quickly close it so as to fill its lumen with urine. Rinse out the larger tube with water and fill it and one-half of the bulb with hypobromite solution, made up fresh by mixing together equal amounts of bromine-water, and 10 per cent sodium hydroxide solution. The stock solutions (A) and (B) suggested by

Rice are satisfactory. Fill urine tube to top of graduation. Turn stopcock and allow 1 cc. of urine to flow slowly into the hypobromite solution. When bubbles cease to rise, read off on the scale which indicates the amount of urea by weight in the urine added. If a very high per cent of urea is present, dilute one-half or if very low add 2 cc. instead of 1 and correct by multiplying or dividing the reading by 2.

Microscopic examination

Preparation of specimens. The objects which we expect to find by microscopical examination are suspended in the urine and must be

Fig. 81. A good type of electric centrifuge for moderate amount of routine work, made by International Equipment Company.

concentrated by means of a centrifuge. Satisfactory examination is impossible otherwise. The electric centrifuge is far the best (Fig. 81). Any one who does his routine laboratory work, as all should do, is poorly prepared indeed to practice medicine without a good centrifuge. It is one of the essential pieces of apparatus for a doctor's office laboratory. It should have a guard (Fig. 82) around it to protect against accidents.

A good hand centrifuge (Fig. 83) does well in the hands of those who take proper care of them and run them right. They do not last long if run with tubes of unequal weight in them or if turned by jerks

at the very highest speed possible. Neither is necessary. The centrifuge should be supplied with square bottom Cornell shields with rubber washers in the bottom. Do not accept any other. No special centrifuge tubes are required. Use the regular 12 mm. by 115 mm. lipless tubes. Agitate the urine to stir up any sediment that may have settled to the bottom of the container and fill the tube about two-thirds full. Select another tube as nearly the same size as possible and fill it with water to exactly the same level (Fig. 84), holding

Fig. 82. Home-made box guard around electric centrifuge

them side by side for comparison. If two specimens are to be centrifugalized, each tube may be filled with urine and (one) labeled.

A centrifuge with a four-tube head permits centrifugalizing four tubes at once. Centrifugalize at full speed one-half to two minutes. Longer is unnecessary, except when endeavoring to throw down bacteria. This cannot be done satisfactorily with the hand centrifuge and requires several minutes with the electric centrifuge. If not sufficient sediment is obtained from the first tube, the supernatant fluid may be poured off, leaving the sediment in the bottom, the tube refilled with urine, and centrifugalized again. In this way all the

suspended material in several tubes of urine may be collected if desired, but generally one tube is sufficient for all practical purposes.

After centrifugalizing, pour off the supernatant fluid. The sediment and a drop or two of urine remain. Shake the tube so as to break and stir up the sediment and pour it out on a slide. Spread out properly on the slide with the edge of the mouth of the tube (Fig.

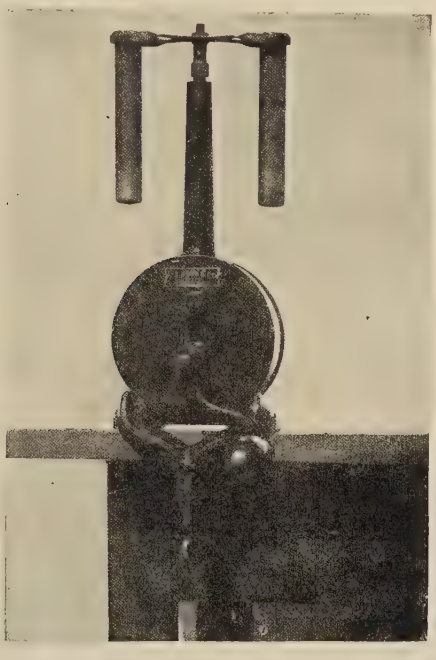


Fig. 83. Hand centrifuge. A very poor excuse for a centrifuge for physicians' use. Note flat bottom "Cornell" shields. These permit use of ordinary test tubes and are essential for best service, whatever the kind of centrifuge used.

85). A proper spread (Fig. 86) will not extend to the edges or end of the slide. It is the height of laboratory awkwardness to allow urine to run over onto the microscope stage, which *will* occur if it is spread to the edge or end of the slide. No cover-glass is necessary for routine examinations.

If examination for minute quantities of pus cells and bacteria is desired, it is better to make and examine a stained preparation.

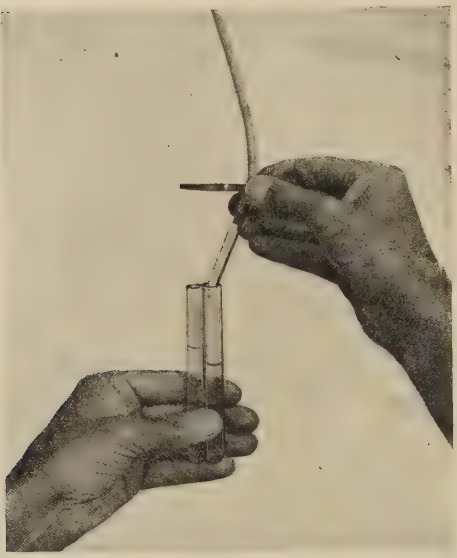


FIG. 84. Tubes held side by side for comparison while filling balance tube to height of column of urine in the other tube.

FIG. 85. Pouring out sediment and spreading on slide with mouth of the tube. No pipette is necessary. The same method is followed in making preparations of sediment from feces.

After centrifugalizing and collecting the sediment in the bottom of the tube, drain off the last drop of urine and with a platinum loop take up the sediment and spread on a slide like other pus. Dry and stain (technic page 128) and examine.

Method of examination. Examine with the low power (16 mm.) lens. It will occasionally be necessary to swing in place the high dry

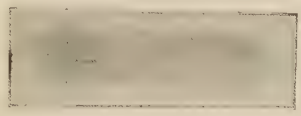


Fig. 86. Proper spread of urine sediment. Note that it does not come to the edges of the slide. This can not be done with greasy slides.

lens (4 mm.) to examine under higher magnification, some particular object about which doubt exists. Here as well as in other places it is important to have a high power lens with a long working distance, such as the B. and L., 4 mm. of N.A. 0.65 (and not one of 0.85) or the spencer 4 m.m. of N. A. 0.66 (and not one of N. A. 0.85), or the Leitz No. 6 l.g. (and not No. 6). Those who, through mistake or

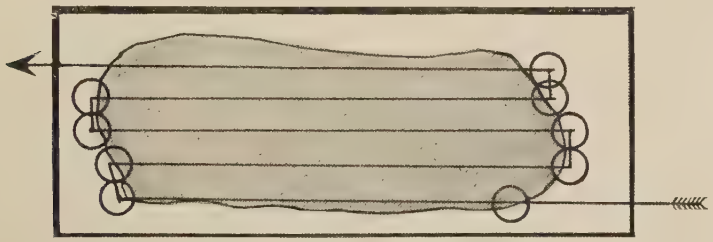


Fig. 87. Diagram indicating proper method of going over the entire preparation in examining preparations of urine, feces, etc. The mechanical stage makes this possible.

otherwise, have purchased lenses with short focal length should exchange them or procure proper ones in some way. Do not try to do routine laboratory work under such a handicap.

Place one corner of the preparation in position for examination and by means of the mechanical stage move the slide from end to end, dropping down the width of a field each time until the entire specimen is examined (Fig. 87). This requires only a minute or two where the examiner is competent.

The light of the microscope must be adjusted (reduced) so that hyaline objects and those with little color may be seen well (Fig. 14).

Diagnosis of microscopic objects commonly found in urine. Red blood cells (Plate V) are usually more or less altered by the urine (Plate X). They may be swollen or faded or crenated. They appear greenish instead of red. They may be differentiated from other objects, when in doubt, by mixing a little acetic acid with the material on the slide. Red blood cells are decolorized while other cells can be seen better.

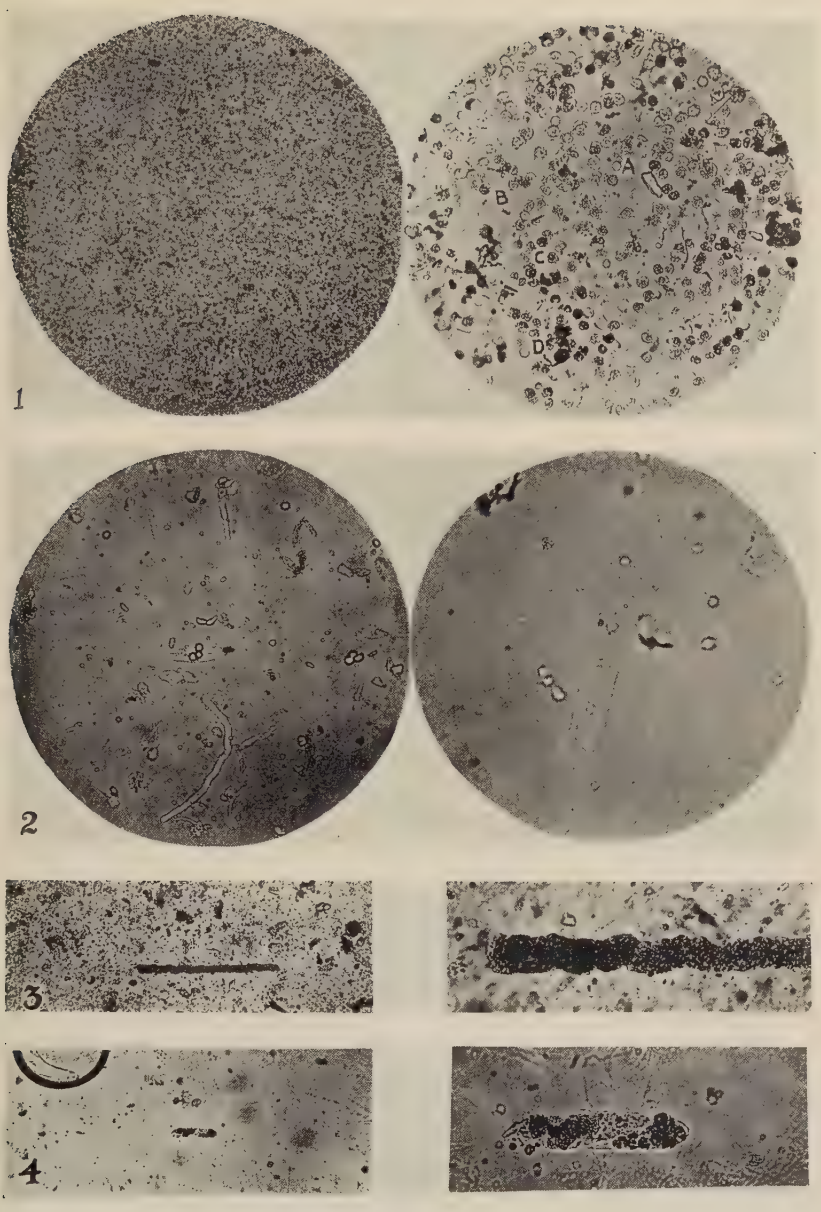
Pus cells (Plate X) are to be recognized by their granular cytoplasm and their nuclei which can be made out upon proper adjustment of the light. When in doubt add a drop of acetic acid to bring out the nuclei better or a suitable preparation may be made and stained (see technic page 128).

Epithelial cells (Plate X) vary much in their size, shape and appearance according to what part or parts they come from. They are to be recognized by their distinct, relatively small, single nuclei.

Casts (Plates X and XI) are of several varieties. They are casts of uriniferous tubules and therefore vary in size and shape according to the size and shape of the different tubules in which they are formed. As the casts are forced out of the tubules, they are often broken so that some are short pieces while others are very long and many others are of intermediate lengths. Some are two or three times as thick as others. The basis of all casts is a hyaline material. Many of them consist of this material only and are called hyaline casts.

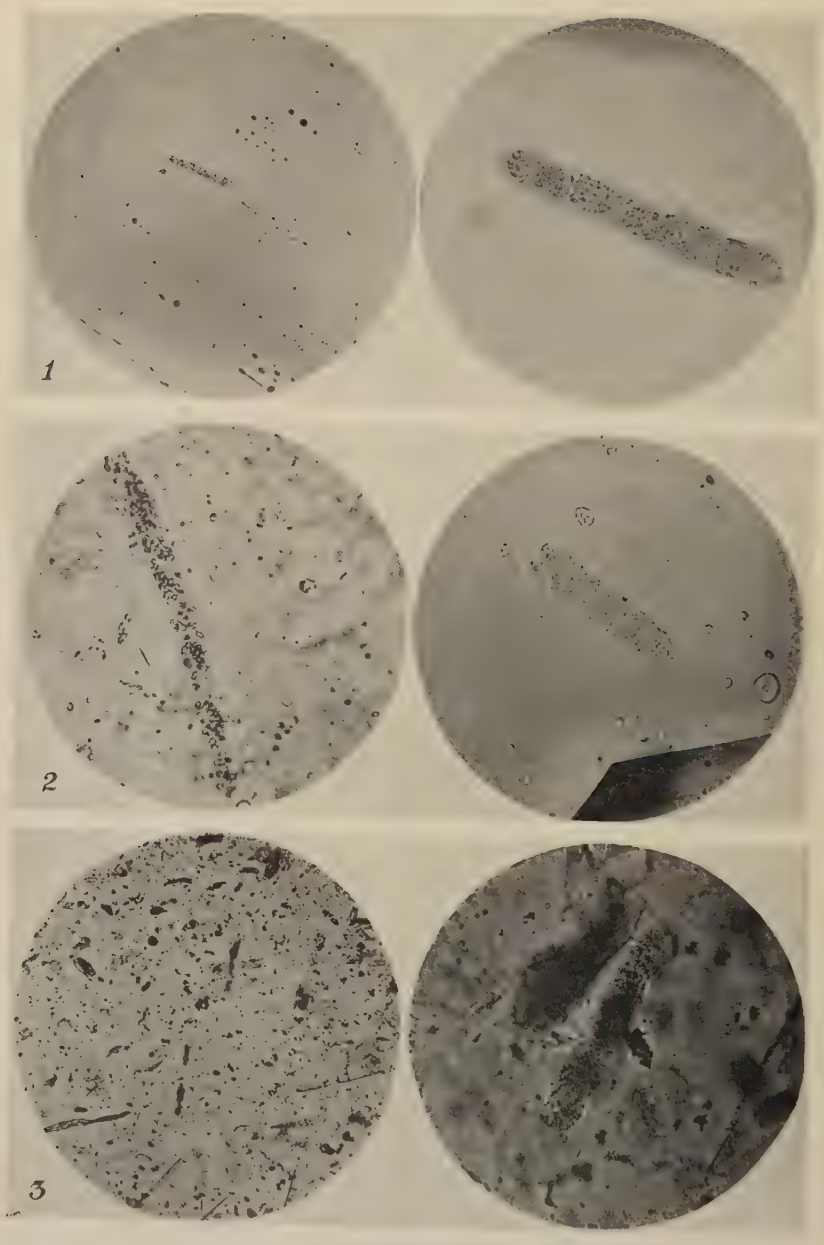
Sometimes the disease involving the epithelial cells lining the tubule where a cast forms results in the loss of the cement substance holding the cells in place and some of them adhere to the cast when it is forced out. These cells are usually swollen and colored more or less yellowish or brownish. Such casts are called epithelial cell casts. An epithelial cell cast is a hyaline cast in which epithelial cells are embedded.

If such damaged cells are not carried out with casts, they may continue to degenerate and break up into coarse granules. These adhere to casts that form in the tubules and such casts are now called coarse granular casts. The granules vary from a light yellow to dark brown in color. A coarse granular cast is a hyaline cast with coarse granules adhering to or embedded in it.



MICROSCOPIC OBJECTS IN URINE

1. Pus, blood and bacteria in urine sediment photographed under low and high dry magnification. *A*, red blood cell; *B*, red cell shadows; *C*, pus cells; *D*, epithelial cell.
2. Hyaline casts. Low and high dry magnification.
3. Fine granular casts.
4. Coarse granular casts.



CASTS IN URINE

1. Epithelial cell cast. Low and high dry magnification.
2. Red blood cell cast (left). Waxy or amyloid cast (right).
3. Mucus cylindroids with adherent amorphous precipitate (low and high).

Unless the granules are carried out while they are coarse, they continue to break up into finer granules and casts forming in a tubule where such a process is going on would have some fine granules on them. In fact, granules are actually imbedded in the casts. Such casts are called fine granular casts. A fine granular cast, therefore, is a hyaline cast with fine granules on or in it.

Sometimes the degeneration of the lining cells of tubules which generally gives rise to epithelial, coarse granular and fine granular casts, is a fatty degeneration instead of granular degeneration, and casts formed in such tubules then have fat droplets on or in them. Such casts are called fatty casts. A fatty cast is a hyaline cast with fat droplets on or in it.

It sometimes occurs that red blood cells pass into tubules where casts are forming and adhere to them. Such casts are called red blood cell casts. Likewise, occasionally pus cells may be attached to casts where they form. Such casts are called pus cell casts.

There are often various combinations of the above different varieties of casts. For instance, a single cast may have epithelial cells, coarse granules, fine granules, and fat droplets on or in it. It often occurs that one end of a cast may be hyaline while the other may be loaded with granules.

Rarely casts consist of a *highly refractile* amyloid or waxy material of light yellowish color instead of the usual hyaline material. Such casts are called amyloid or waxy casts.

In addition to the above true casts there are present in most fresh specimens of urine a variable number of shreds or cylindroids (Plate XI) of a mucoid material very much resembling hyaline casts. They are irregular in shape and size and one end is usually very thin. They are much narrower in places than in others and do not give one the impression that they are true casts of the uriniferous tubules. The dividing line between hyaline casts and some of these cylindroids is difficult to draw. In fact, one must often feel in doubt about some of them. When in doubt, give the patient the benefit of the doubt.

Interpretation of urine examinations. The normal specific gravity varies from about 1.015 to 1.025. It is high (above 1.025) in most cases of glycosuria and very low in various nervous conditions. A low specific gravity (1.000 to 1.010) with inability to concentrate occurs in "chronic interstitial nephritis." Normal values or higher occur in different forms of nephritis with edema. The specific gravity

is susceptible of great variation in health according to the amount of water taken in and the amount of solids excreted.

The reaction of normal urine is weakly acid. Alkaline urine results from an almost exclusive carbohydrate diet. Decomposition of urine, either in the bladder or after it is voided, usually changes the reaction to alkaline. Strongly acid urine is produced by a preponderance of protein or fats in the diets.

Albumin in urine is derived from the blood serum and when present indicates that on account of some influence, serum and other proteins are passing through the kidneys. High blood pressure often causes a small amount of albumin in the urine. Nephritis is the chief cause of albuminuria, the amount of albumin varying greatly with the different forms of nephritis and in different individuals. Albumin in the urine is also produced by inflammatory disease accompanied by suppuration involving the mucous membrane of any part of the urinary tract. The albumin is derived from the inflammatory exudate and naturally varies in amount greatly.

Sugar (glucose) in the urine results from diseases and conditions involving the metabolism of carbohydrates. Excessive eating of sugar may produce it temporarily in individuals who are apparently healthy. Diseases and injuries involving the floor of the fourth ventricle, pancreatitis and hepatitis, are usually accompanied by a mild glycosuria. The chief cause of glycosuria is diabetes mellitus, a disease of the pancreas involving the islands of Langerhans.

An excess of indican in the urine indicates absorption of products of putrefaction of proteins in the alimentary tract. Intestinal stasis or obstruction is an important cause.

Acetone in the urine indicates excretion by the kidneys of end-products of metabolism of the body fats whenever there is not sufficient carbohydrate available in the tissues. In severe cases of acidosis the fats are split into di-acetic acid.

Di-acetic acid indicates an advanced stage of deficient carbohydrate metabolism and burning of body fats. It is always preceded and accompanied by acetonuria. Therefore, the presence of acetone is a good check on the accuracy of the test for di-acetic acid.

Bile indicates an obstruction to the flow of bile somewhere between the bile radicals and the opening of the common bile duct.

Urobilin is one of the pigments contained in bile. In hepatitis the excretory function of the liver is interfered with and the increased

accumulation of this pigment in the blood stream results in its excretion into the urine.

Red blood cells in the urine show that hemorrhage is going on somewhere along the urinary tract. The presence or absence of erythrocytes in the urine is very valuable in differentiating "renal colic" from other pain due to disease in other organs in the same region. They are also present in hemorrhagic nephritis.

Pus cells in urine indicate suppuration somewhere along the genito-urinary tract. There are a few or many pus cells from the vagina and vulva in voided specimens of urine from most women. Pus found in urine should never be considered normal, even though it be merely a vaginal contamination. Many pus cells would at least indicate a purulent leukorrhea.

Epithelial cells are present in all specimens of urine. Many more are present in voided specimens from females.

A few hyaline casts are present in the urine of most people past the age of fifty, especially if the blood pressure is high. A few may also be found in the urine of persons who have not any recognizable disease. Hyaline casts, therefore, do not necessarily indicate any severe disease of the kidney. On the other hand, they are present and usually in large numbers in nephritis of all types.

Epithelial and coarse granular casts indicate acute nephritis. Fine granular casts are present in all forms of parenchymatous nephritis, but generally speaking they indicate somewhat less acute disease than the coarse granular and epithelial cell casts indicate.

Pus cell casts indicate production of pus in the tubules.

Red blood cell casts indicate that blood cells are leaking through the walls of the tubules at the time and at the place where casts are being formed.

Cylindroids have no definite pathological significance.

Bacteria in ordinary specimens of urine have little or no significance. Proper precautions should be taken to avoid contamination of specimens and make sure that any bacteria found come from the bladder or kidney. When mixed with pus cells, especially if some of these contain phagocyted bacteria, this is strong evidence of bacterial inflammatory process. Bacteria with pus cells in specimens catheterized from the ureters, if very numerous, indicate pyelitis. Many an incorrect diagnosis of pyelitis is made on the presence of a few pus cells and bacteria in voided or even catheterized contaminated specimens.

CHAPTER IX

Gastric Contents

Obtaining material to be tested. After the patient has fasted twelve hours (over night) or longer, give a test meal consisting of two or three crackers or a slice of bread and one or two glasses of water. The food should be thoroughly masticated. After about one hour remove the gastric contents with the stomach-tube. A tube with a bulb to make suction with is quite an advantage.

The Rehfuss tube is far superior to the ordinary stomach tube for removing gastric juice. It consists of a slotted steel or bronze, silver-plated bulb, to which a No. 8 French tube is attached. The metal tip is placed well back in the patient's pharynx and he is directed to swallow several times in succession. Deep breathing aids in overcoming nausea. When the bulb has reached the stomach, indicated by the tube going down as far as the mark on it for this purpose, gastric contents is obtained by aspirating with a glass syringe. A syringe of 20 cc. capacity is most convenient, but one of 10 cc. capacity is satisfactory.

Test for free HCl and total acidity. Obtain some clear gastric juice by centrifugalizing a tube of the gastric contents just removed. The heavy particles go to the bottom. The froth, etc., rises to the top. Remove 1 cc. of the clear fluid with a graduated pipette (1 cc.) and place in another test-tube. Add one drop of a 0.2 per cent alcoholic solution of dimethylaminoazobenzol. If free HCl is present a bright cherry red color (Plate XI, 10) will appear at once. Now add one drop of 0.2 per cent solution of phenolphthalein in alcohol. No considerable change occurs. It is now ready for titration with decinormal alkali solution, the dimethylaminoazobenzol serving as an indicator for the free HCl and the phenophthalein for the total acidity.

Now take up in a 1 cc. pipette, graduated in hundredths, 1 cc. of decinormal sodium hydroxide solution. Add this slowly to the fluid, shaking as each 0.1 cc. or less is added. Note the amount of alkali required to just cause the disappearance of the cherry red color

TEST OF GASTRIC JUICE FOR FREE HCl AND TOTAL ACIDITY

TUBE 9. Clear gastric juice.

TUBE 10. Change of color, upon addition of dimethylaminoazobenzol, indicating free HCl.

TUBE 11. HCl has been neutralized by the addition of decinormal sodium hydroxide solution. There is usually a yellowish tint, due to organic acids.

TUBE 12. Neutralization of all acids (total acidity). Color produced by phenolphthalein indicator.

(Plate XII, 11). This indicates the amount of free HCl present. Continue to add the alkali solution until a permanent rose color (Plate XII, 12) appears. This indicates the total acidity and the total amount of alkali used should be noted. The degree of acidity is expressed in terms of the number of cubic centimeters of the decinormal solution that would be required to neutralize 100 cc. of gastric fluid. Each one hundredth cubic centimeter of the decinormal solution in our test represents one degree. For instance, if 0.35 cc. of the decinormal solution is required in the first titration, the total free HCl would be 35, and if 0.65 cc. (in all) is required before the second reaction is obtained, the total acidity would be 65.

Test for lactic acid. To test for the presence of lactic acid, fill a test-tube about two-thirds full of water, add a few drops of ferric chloride solution sufficient to produce a light yellow color and then add to the top a few drops of the gastric juice to be tested. The clear fluid obtained by centrifugalization, advised above, or by filtration is best for this purpose. If lactic acid is present in an appreciable amount, the light yellow color is intensified to a deep yellow or deep brown according to the amount of the acid present.

Other examinations. Test gastric contents for occult blood in the same way as feces are tested for it (see page 123).

Blood and other cells, food remnants, etc., can be recognized by microscopic examination. Look first at a specimen of the material, preferably the sediment, with the high dry objective. The addition of a drop of Lugol's iodine solution to a few drops of sediment is helpful. Muscle fibers, yeast cells, red blood cells and epithelial cells are stained a golden yellow; starch granules are stained blue, while fats are unstained and show as highly refractile globules. If in doubt as to the diagnosis of pus cells, make a spread on a slide, stain (technic, page 128), and examine with the oil immersion lens. Boas-Oppler bacilli are recognized in stained specimens by their great abundance, morphology and Gram-positive staining.

Interpretation of the findings in gastric contents. The free HCl in gastric contents of normal individuals is usually between 25 and 50, by this test. Above 50 indicates hyperchlorhydria and occurs in neuroses, most cases of gastric ulcer and beginning chronic gastritis. Values below 30, hypochlorhydria, are usually found in early carcinoma and in most conditions associated with general systemic

depression, including some neuroses. Absence of free HCl, achlorhydria, occurs in most cases of advanced gastric cancer, pernicious anemia and far advanced chronic gastritis. Total acidity includes free HCl, organic acids, combined acids and acid salts. Values are always somewhat higher than for free HCl.

Lactic acid is not normally present in gastric juice. It may be taken with food chiefly in some form of sour milk and this should not be overlooked in interpreting its presence. It may be formed in the stomach by fermentation of the food by lactic acid producing bacteria, chiefly Boas-Oppler bacilli, which grow abundantly in the absence or near-absence of HCl. Lactic acid and Boas-Oppler bacilli are abundant chiefly in advanced carcinoma of the stomach.

Absence of occult blood is strong evidence against carcinoma of the stomach. Its presence tends to support other evidence of carcinoma, but is of little or no significance when there is no other evidence, for the reason that positive findings may result from trauma in passing the tube and extraneous or other sources of small amounts of blood.

CHAPTER X

Feces

General considerations. The normal stool consists chiefly of undigested food remnants, various products of digestion and enormous numbers of bacteria, most, if not all, of which are of non-pathogenic varieties. In fact, a large part of the total volume of feces is made up of bacteria, dead or living. Food remnants are greatly increased in diseases in which digestion is impaired and in those accompanied by diarrhea, for the reason that food material is swept out before digestion and absorption are complete. Many of these food remnants can be recognized and identified either macroscopically, such as seeds, berry, fruit or vegetable skins, etc., or microscopically, such as muscle fibers, vegetable cells, fibers, hairs, etc. The quantity of the feces is influenced largely by the amount and character of the food. A vegetable diet increases the amount. The amount for a normal adult is about 200 grams per day.

Soft, mushy, or liquid stools follow cathartics and accompany diarrhea. Profuse watery stools often accompany acute digestive disturbances, especially in children, and such diseases as Asiatic cholera. Putty-colored or "acholic" stools occur when bile is deficient, either from deficient secretion or from obstructed outflow. Pancreatic deficiency gives rise to greasy stools.

Large, foamy, soft, acid stools occur especially in sprue and in other conditions accompanied by carbohydrate indigestion.

Large amounts of blood produce tarry-black stools when the source of the hemorrhage is high up and dark brown to bright red where the source is near the rectum.

Examination for intestinal parasite ova and larvae. All of the intestinal worms either lay eggs or deposit larvae in the intestinal canal. These may be found in the feces. Most of the worms produce very large numbers of ova or larvae. They are characteristic of the particular kind of worm that produces them. Whether a given individual is the host of intestinal worms can therefore be determined by microscopic examination of the feces for ova and larvae. The kind of worms present can also be determined by the same means.

Collection of specimens. All of the intestinal worms commonly found in this country inhabit the small intestine and cecum, except trichiura and oxyuris, which inhabit the large intestine. The ova and larvae being deposited high up in the intestine are thoroughly distributed in the feces. Oxyuris ova are sometimes more numerous on the surface of formed stools. Feces of normal consistency are most suitable for examination. Liquid feces generally contain fewer ova, but they usually contain a sufficient number for diagnostic purposes.

FIG. 88. Proper specimen bottle containing formed feces. Quantity is sufficient. Bottle should not be full.

One or 2 drams of feces is sufficient quantity. Larger quantities are objectionable and sometimes unpleasant and embarrassing. Unless patients are instructed as to the quantity desired and how to collect it, they sometimes bring to the laboratory very large quantities and in the most inappropriate containers. It is not at all uncommon to have a fruit jar full of liquid feces brought to the laboratory for microscopic examination; and an unwieldy package it is. Or a bottle is completely filled with fermenting feces and tightly stoppered. The shaking during transportation increases gas formation, and whenever the stopper is loosened it often shoots out, scattering feces about the laboratory and sometimes on the examiner. A wide mouth, 2-ounce bottle (Fig. 88) with a new cork stopper is a very

appropriate container. The patient may be instructed to obtain such a bottle from the drug store or elsewhere, or it should be furnished him at the time the specimen is requested. At the time the container is supplied and the specimen is requested, it is well to tactfully tell the patient that *only a small quantity*—"about so much"—

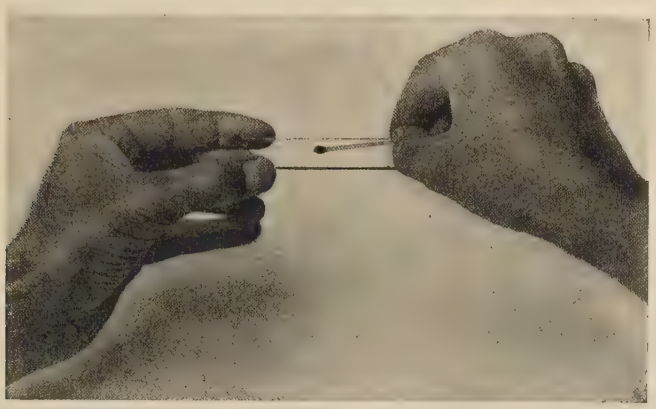


FIG. 89. Stirring feces in a drop or two of water on a slide to make properly diluted preparation for direct microscopic examination.

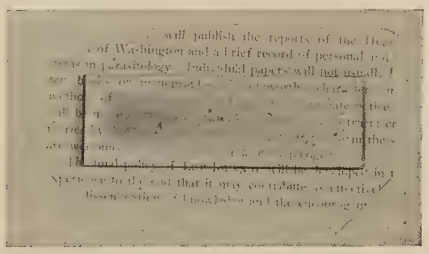


FIG. 90. Proper spread of diluted feces. Note that ordinary print may be just read through it. Thicker preparations cannot be examined microscopically.

is desired, and *not to fill* the bottle full. Specimens should be submitted for examination within twenty-four hours of the time they were passed whenever practical, to facilitate the recognition and differentiation of strongyloides larvae, but much older specimens are satisfactory to examine for ova.

Making the preparation for examination. Place two drops (not

more) of water in the middle of a slide. Take up a quantity of feces about the size of a match head, on the end of an ordinary wooden toothpick. Stir the feces (Fig. 89) in the water on the slide until sufficient has been dissolved to produce the proper concentration of feces for examination. The material should be properly spread out on the slide at the same time. Do not let it come to the edge or end

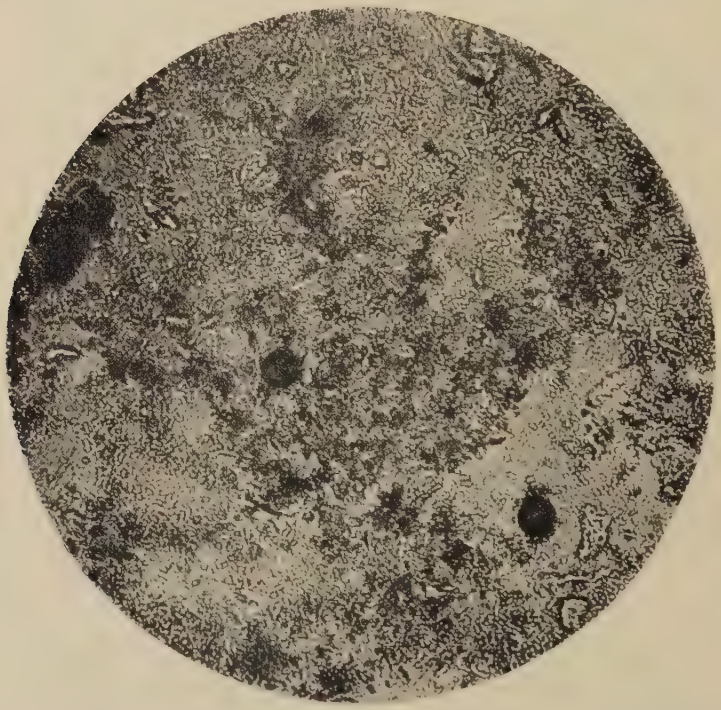


Fig. 91. Photomicrograph of feces prepared in the ordinary way, showing two tapeworm ova.

of the slide (Fig. 86). The solution of feces should be about as thick as will just barely permit ordinary print to be read through it (Fig. 90). It is not necessary to dissolve or use all the feces taken up on the pick. Return the soiled pick with any undissolved feces on it to the bottle or dispose of it in any other convenient way. No cover-glass is required. The preparation is ready for examination.

Concentration of ova by means of the centrifuge. All the ova, or at least nearly all of them, in a drachm or two of feces may be collected by proper use of the centrifuge and placed on one side. Considerable concentration of the ova (Figs. 91 and 92) may thus be obtained, and whenever very small numbers are present this very much facilitates

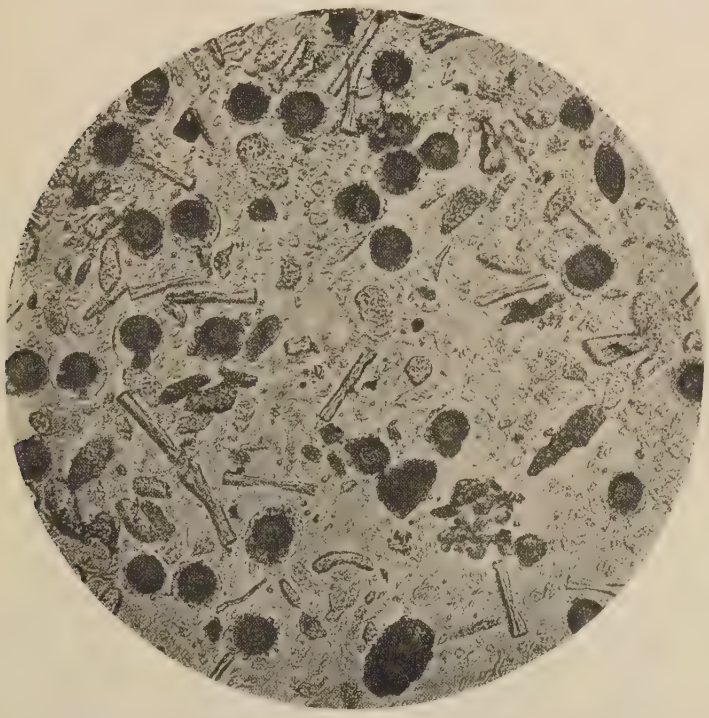


FIG. 92. Photomicrograph of preparation from the same specimen of feces shown in Fig. 91, made by centrifugalizing as directed on page 113. This shows great concentration of the tenia ova and also the presence of ova of trichiura and ascaris which might be overlooked in examining a preparation not made in this way.

the search for them. No examination should be considered finally negative until after this method has been carried out. It should be employed, however, only after at least one or more slides, prepared in the ordinary way, have been examined.

Add some water to the feces in the bottle. Shake until a proper suspension (Fig. 93) is obtained. The feces should be diluted about

FIG. 93. Solution of feces made in the bottle in which it was brought to the laboratory.

FIG. 94. Straining diluted feces through gauze directly into centrifuge tube

ten times. After the suspension is of about the proper consistency stop shaking it and do not dissolve the remaining feces. Now strain into a centrifuge tube through two or three layers of gauze placed in a small glass funnel (Fig. 94). This removes any large coarse particles. Now centrifugalize (Fig. 95) for only a sufficient length of time to throw ova or larvae to the bottom of the tube. This depends upon the speed of the centrifuge and other factors governed by the particular centrifuge used. The length of time required by any

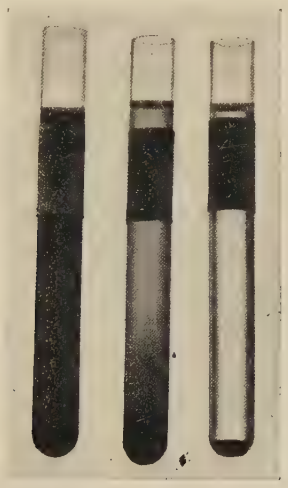


FIG. 95. Diluted feces after centrifugalization. Tube I, diluted feces after first centrifugalization. Tube II, after second centrifugalization. Tube III, after third centrifugalization. Note that the supernatant fluid in this tube is almost clear.

particular centrifuge may easily be determined by experiment with a specimen known to contain a good many ova. A good electric centrifuge (Fig. 81) requires only about four to eight seconds to throw ova to the bottom of the tube. Encysted amebas require only a few seconds longer. A hand centrifuge (Fig. 83) requires longer time, but may be used.

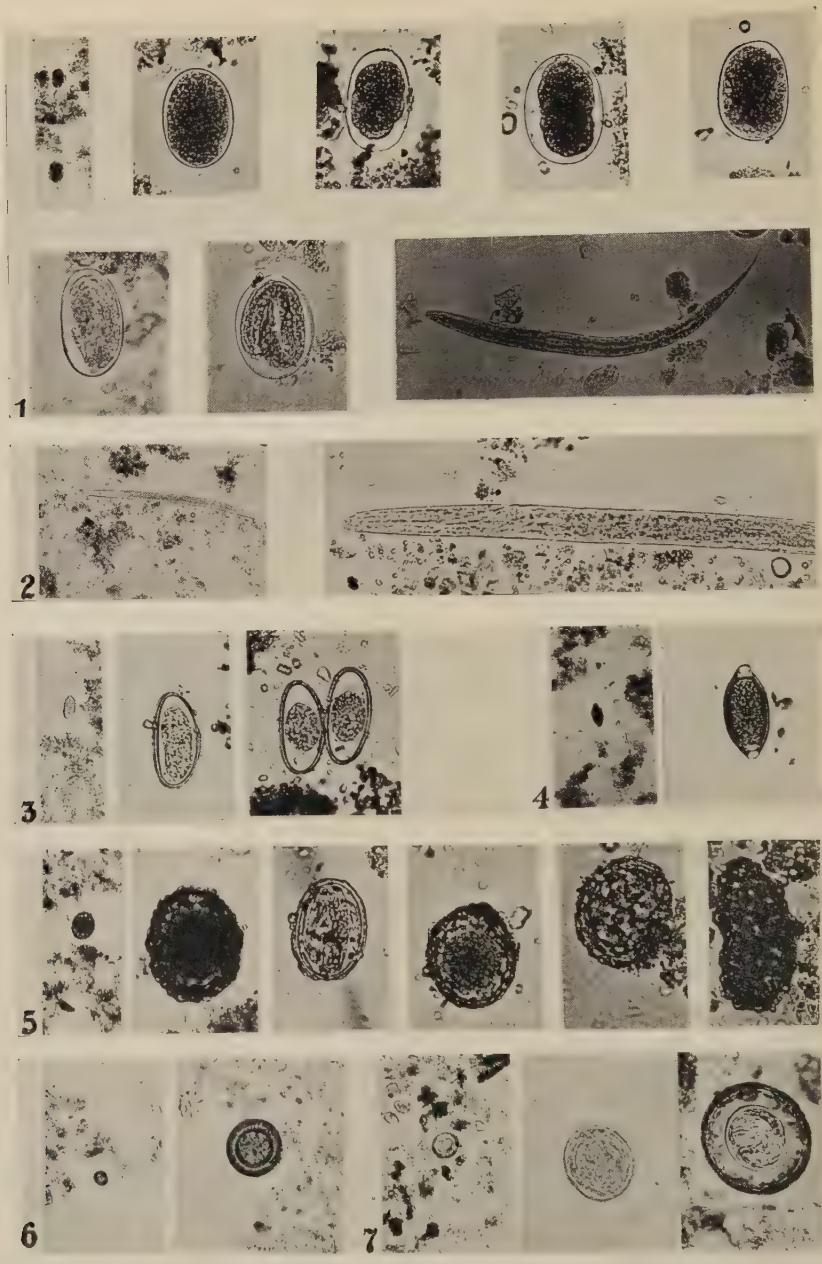
The diluted feces consists of a suspension of particles most of which are much smaller than ova. In fact, the bulk of normal feces is bacteria. These small particles go down more slowly than the larger,

heavier ova. It is important, therefore, to run the centrifuge just long enough to throw the ova to the bottom, and to *stop before the smaller particles are thrown down.* Pour off all the contents of the tube except the sediment in the bottom, which should contain the ova. Refill the tube with water to the former level, shake thoroughly and centrifugalize again. Note that the supernatant fluid is not so cloudy this time. Pour it off, as before. The sediment, which still contains any ova that were in the original suspension of feces, may be

Fig. 96. Pouring out sediment from feces on slide and spreading with lip of tube.

washed again and again until it is as clear as desired, of the smaller particles of which feces are mostly composed. Usually two or three washings are all that are necessary. After pouring off the supernatant fluid there remains the sediment, and at least a drop or two of fluid. Shake the tube, so as to stir up the sediment, and pour it out on a slide (Fig. 85), spreading it properly with the test-tube (Fig. 96). It is now ready for examination.

Method of examining a slide preparation. The examination is made with the low power objective (16 mm.). The light must be adjusted so that objects with little or no color may be seen plainly (Fig. 14).



PHOTOMICROGRAPHS OF OVA AND LARVAE OF PARASITIC WORMS

1. Ova and larvae of Uncinaria americana (low and high dry).
2. Larva of Strongyloides intestinalis.
3. Oxyuris vermicularis.
4. Trichuris trichiura.
5. Ascaris lumbricoides.
6. Taenia saginata.
7. Hymenolepis nana.

Try the effect of opening and closing the diaphragm until the best light is obtained. *See that the light is properly adjusted* for each specimen. The light may require changing from time to time while looking at a given specimen.

Begin at the corner (Fig. 87) of the preparation. By means of the mechanical stage move toward the opposite end of the slide, looking for ova and larvae as the slide is moved. Whenever the end of the preparation is reached drop down the width of the field and move in the opposite direction. Upon reaching the end, drop down the width of the field as before, and so continue until the entire preparation has been examined. In this way the entire spread of material may be looked over in a few minutes. After one has acquired experience, the slide may be moved quite rapidly, only hesitating or stopping now and then to focus on and examine more closely suggestive objects that come into view. Once in a while it may be necessary to move in place the high power objective (4 mm.) to study more closely, under higher magnification, some doubtful object, but this lens should not be used for routine examination. The field covered by this lens is so small that it would take entirely too much time to look over a given preparation.

Uncinaria ova. Uncinaria ova (Plate XIII) are oblong, round-ended, and measure about 38 microns in diameter and 60 microns in length. They consist of three distinct parts—the shell, the nucleus or yolk, and the clear space filled with albumin between the yolk and the shell. An object should not be diagnosed as an ovum unless these three separate parts can be made out.

The shell appears as a distinct narrow line. It is regular in outline. The nucleus is made up of granular material which appears gray or dark gray, according to the light used. The nucleus usually shows a certain amount of development varying from division into two segments up to embryo formation. Most ova, however, in fresh specimens are in the two to eight segment stage. The nucleolus, a clearer spot, can usually be recognized in each segment. The space between the shell and the nucleus is of diagnostic importance. The nucleus is irregular in outline, according to the stage of division, but the shell remains regular. This is in contrast to certain vegetable cells and other objects which have an outer membrane and a nucleus, suggesting ova. In these objects the outer membrane

dips in wherever there are indentations in the nucleus, and this gives the object an irregular outline. They also vary in size considerably, while the size of ova of any given kind of worm is fairly uniform.

Ascaris lumbricoides ova. The ova of the ascarides (Plate XIII) that infect man vary considerably in size and shape. They measure about 64 by 52 microns. The outer membrane proper is covered with a thick layer of gelatinous material, which is very irregular in outline and gives the ovum a very rough nodular appearance. The color of this varies from a very light to a very dark brown. Ova are sometimes found from which this outer coat has been partially or completely torn away.

The nucleus of ascaris ova cannot be seen as well as the nucleus of the clear ova, largely because of the thick colored outer coating. However, upon proper adjustment of the light, a clear space can always be made out between the shell proper and the nucleus.

Trichuris trichiura ova. These ova (Plate XIII) are very characteristic in appearance and should never be mistaken. They are oblong, measure about 52 by 25 microns and vary in color from light brown to very dark brown. There is a knob of lighter-colored material on either end. The nucleus and clear space can be made out in most specimens, but the thick, dark-colored shell prevents their being seen as satisfactorily as in the case of some of the more transparent ova—uncinaria, for instance.

Oxyuris vermicularis ova. These ova (Plate XIII) are flattened on one side and oval on the other. They measure about 50 by 30 microns. The shell is thicker than that of uncinaria ova, and is shiny in appearance. The nucleus and clear albumin space are plainly seen. Empty egg-shells are often found. They contain a small mass of bacteria or débris in place of the nucleus. Larvae are sometimes found, and are to be recognized by the long, sharp, pinlike tail.

The eggs of oxyuris are deposited in the rectum and in the folds of the anus and vulva. Therefore, much larger numbers of eggs can be found in material properly collected from these parts than in the feces. A good way to collect material for this purpose is to scrape the surface of the parts with some suitable instrument, like, for instance, the end of a microscope slide. Dilute the material collected with a drop of water on a slide and examine. Both ova and larvae are usually found.

Hymenolepis nana ova. These ova (Plate XIII) measure about 48 by 36 microns. They have an outer and an inner membrane, each of which is quite thin and appears as a distinct line. The space between the two membranes is clear, except for a few striae, which run irregularly longitudinally. The nucleus is light gray and contains 4 to 6 very distinct hooklets. The space between the inner membrane and the nucleus is not very large and looks clear.

Tenia saginata and Tenia solium ova. These ova (Plate XIII) are oval, brownish or grayish colored, and measure about 36 by 30 microns. There is an inner and an outer membrane, and the space between contains a great many radial striations. On account of the

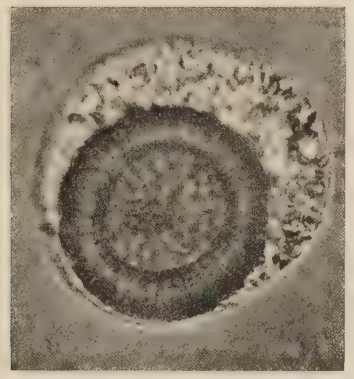


FIG. 97. Photomicrograph of ovum of Tenia saginata enclosed within its vitelline membrane. This membrane is usually soon lost after the ova are expelled into the feces, and most specimens do not show it.

color of the covering the nucleus is not so well seen. Hooklet-like objects are present in the nucleus. These ova are sometimes seen covered by a vitelline membrane (Fig. 97).

Larvae of uncinaria and strongyloides. After feces containing uncinaria ova have been out of the body for twenty-four hours or more some of the ova are likely to hatch if the conditions are favorable. The larvae (Plate XIII) are about 0.21 mm. long by 0.02 mm. thick. They wiggle about for a while, but usually die in undiluted feces within a few hours. In this stage they are known as *rhabditiform* larvae. They look very much like the rhabditiform larvae of strongyloides.

The ova of strongyloides are deposited by the adult worm in the glands of Lieberkuhn, and there hatch, giving rise to rhabditiform larvae (Plate XIII), which are passed in the feces. They wiggle about actively, but often many of them die after a few hours. They are about the same size as uncinaria larvae, and look almost exactly like them.

The most practical differential points between uncinaria larvae and strongyloides larvae for one not especially familiar with these parasites are:

1. Whenever uncinaria larvae are present there are also many uncinaria ova present. Strongyloides larvae are not accompanied by the presence of ova except in cases of double infection, in which ova of the accompanying parasite are present.

2. Uncinaria larvae are not present in feces under twenty-four hours after they are passed. Strongyloides larvae are present from the first.

3. The buccal capsule of strongyloides larvae is very short, while that of uncinaria larvae is relatively long.

Interpretation. Ova and sometimes larvae of intestinal parasites (worms) are present and demonstrable upon proper examination in practically all cases and in all specimens. When only very small numbers of such parasites are present, specimens of feces may sometimes have so few ova that none may be found from a single examination. Although a single negative examination does not positively eliminate the possibility of very small numbers of worms being present, it does eliminate the probability of their being present in sufficient numbers to cause disease.

Examination for amebas. A large part of the cases of dysentery occurring in this country, and especially those occurring in the tropics, are due to pathogenic amebas. There are many carriers of the pathogenic amebas with ulcerative lesions of the cecum who may not at the time of examination have any dysenteric symptoms.

There are many species of amebas in nature, a few of which live as parasites in other animals. They are therefore known as endamebas. Some four or five species are found in man, the most frequent being Endameba coli, which is non-pathogenic, and Endameba histolytica, which is the chief pathogenic species and the chief cause of amebic dysentery. For practical purposes it is important to differ-

entiate between the pathogenic and the non-pathogenic and of less importance to identify all of the various species, which requires more experience and knowledge of protozoology.

Collection of specimens for examination. It should be understood that in amebic dysentery, which consists chiefly of ulceration of varying degree and varying extent in the colon, the amebas are present and reproducing in and on the ulcerating tissue. There is more or less blood and mucus being thrown off from the diseased tissue all the time, and this contains the amebas which we desire to find. During a period of acute active disease there may be many discharges daily, consisting chiefly of small amounts of mucus, usually containing more or less blood. This is the proper material for examination. Cases of acute dysentery can produce such actions by making the effort at almost any time, and furnish upon request the proper fresh specimen for examination.

In the subacute or quiescent stage there may be only an occasional particle of the bloody mucus mixed with the fecal stool. By picking carefully one can usually find satisfactory material in soft loose stools from dysentery patients or in stools following a saline purgative. Some prefer material of this kind. Material scraped with a small, blunt spoon curette directly from the ulcers through a proctoscope is very rich in amebas and is ideal for examination.

The examination of stools for amebas should be made within one or two hours of the time the material was discharged, as vegetative forms rapidly die and disintegrate upon prolonged exposure to air. During cold weather the specimen must be examined even more promptly, unless it is kept warm artificially in some way. It is always better, whenever practical, to have the patient come to the laboratory and there pass the stool into a pan or other vessel or a specimen should be collected through the proctoscope for immediate examination. If this is not practical, another good way is to bring the microscope to the patient.

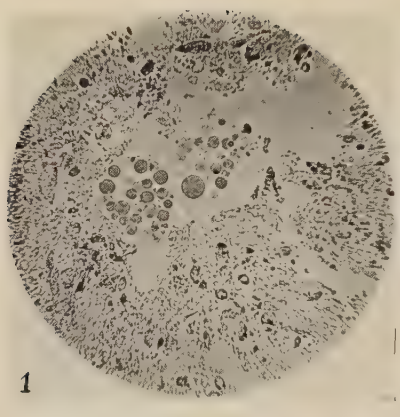
Amebas may be demonstrated in the chocolate colored, purulent material from abscesses of the liver and other parts of the body. They are found present in largest numbers, however, in material scraped from the walls of such abscesses, or in the drainage fluid several days after operation.

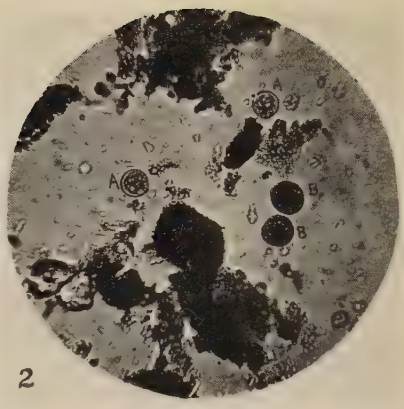
Examination of unstained material. With an ordinary wood tooth-

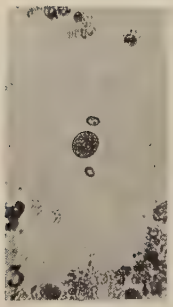
pick held in each hand, pick out particles of the bloody purulent mucus or other selected material and place on a slide. It is better to pick small amounts of promising material from different parts of the stool than to use a large amount from one part only. Put a cover glass on the material on the slide and make slight pressure, if necessary, to thin it out, but do not make too thin. The specimen should be examined with the low power (16 mm.) objective, using the high dry (4 mm.) objective only to examine more closely under higher magnification doubtful objects or for special study of amebas located with the low power. The light must be reduced until the hyaline objects on the slide are clearly seen. Pus and blood cells are readily recognized. Pathogenic amebas (Plate XIV) appear to be larger cells containing coarse granules and blood cells in the endosarc, surrounded by a clear ectosarc. In active individuals the ectosarc is constantly changing its shape. It may throw out a pseudopod into which the endosarc flows. Another pseudopod may be projected in another direction, and either be withdrawn or the ectosarc moves into it. These movements (Fig. 98) are slow and are known as ameboid motion. The Endameba histolytica usually moves rapidly and progressively, and frequently moves out of the field, whereas Endameba coli, the non-pathogenic species, seldom or never moves about to any such extent.

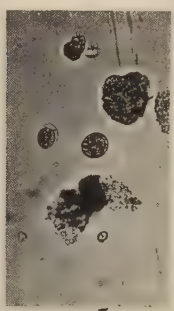
Differentiation of pathogenic from non-pathogenic amebas. The nonpathogenic amebas found in feces are on the average smaller than the pathogenic species and are more sluggish in their motion. There is no sharp differentiation between their ectosarc and endosarc, and they contain numerous bacteria. As mentioned above, many of the pathogenic amebas found in acute dysentery contain numerous erythrocytes, while the others do not, except possibly occasionally. For practical purposes this is the best differential point, and the only one necessary with the vegetative forms. Amebas containing blood cells may, for practical purposes, be considered the pathogenic variety.

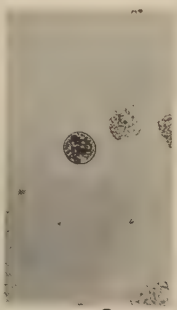
Examination for and differentiation of cysts. Encysted amebas are found in feces of infected persons during intervals between attacks and also in subacute infections or in apparently healthy carriers without active symptoms of dysentery at the time. Cysts of Endameba histolytica are often found in such persons as well as cysts of

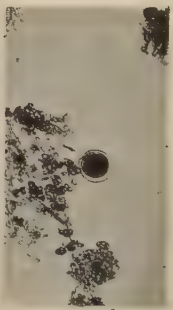


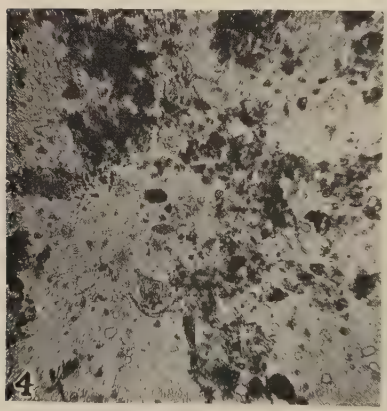


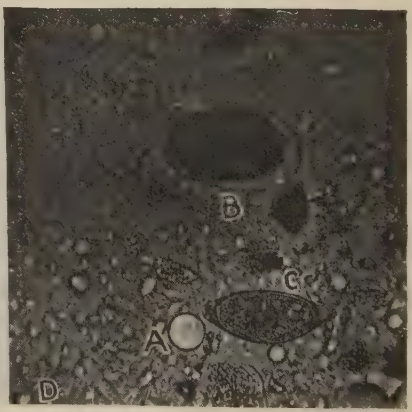












PHOTOMICROGRAPHS OF VEGETATIVE AND ENCYSTED AMEBAS AND INTESTINAL PROTOZOAN PARASITES

1. Vegetative forms Endamebae histolytica.
2. Encysted amebas. *A*, E. histolytica; *B*, E. coli.
3. Cysts of Endamebae histolytica: *a*, mononucleate stage; *b*, binucleate state with cyst of Giardia intestinalis to the left; *c*, four nucleate stage; *d*, dead cyst—cyst wall.
4. Photomicrographs showing relative sizes of worm ova and ameba cyst taken with low and high dry magnification. *A*, E. coli; *B*, uncinaria; *C*, trichuris; *D*, yeast.

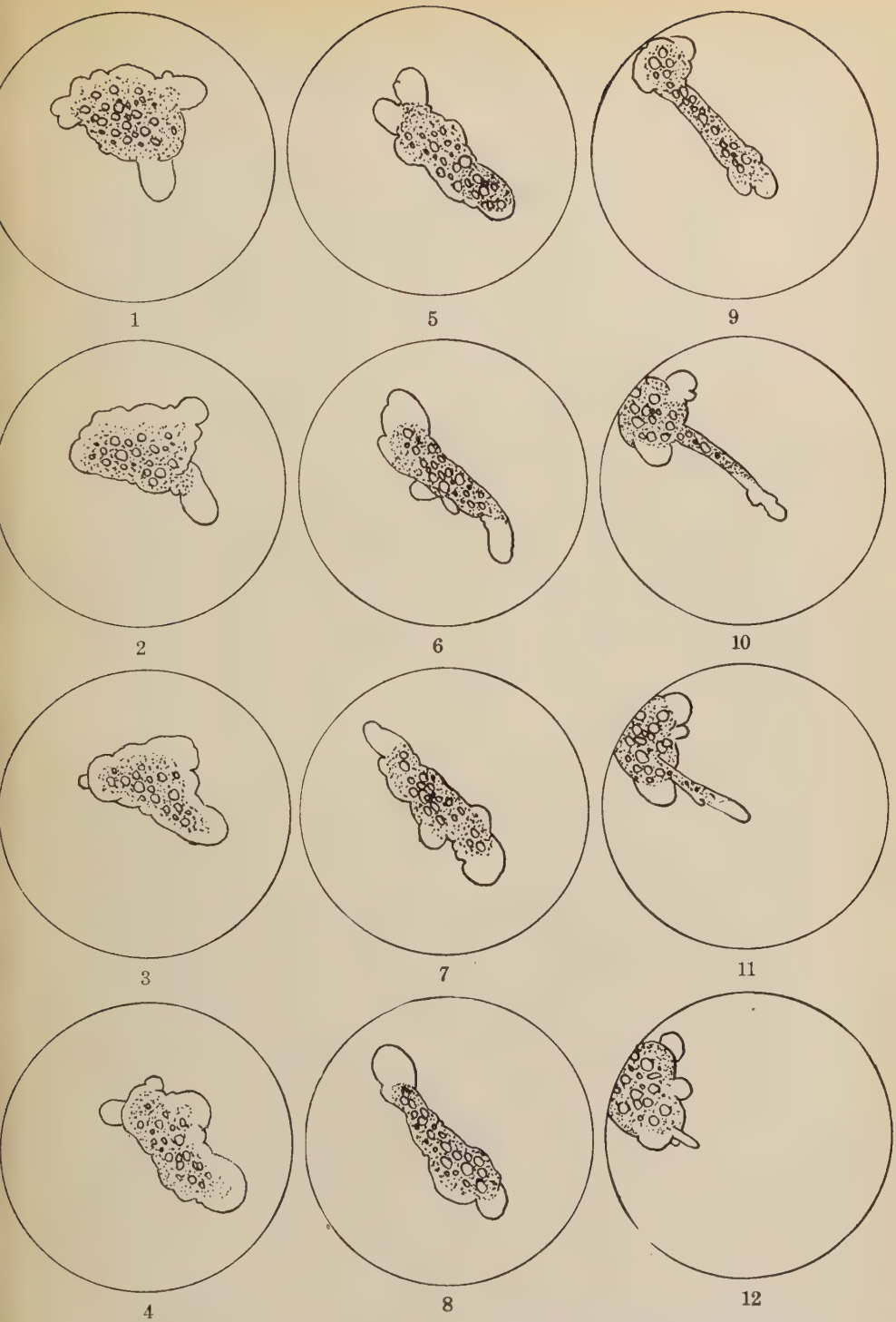


FIG. 98. Changes in shape and position of an endameba during intervals of a few seconds each—ameboid motion.

Endameba coli or other non-pathogenic amebas. They are found most numerous in formed feces.

The routine preparation for examination for ova of intestinal worms is suitable for examination for cysts. In fact, one should form the habit of noting the presence or absence of cysts when looking over preparations for ova. Either the simple preparation of diluted feces or the centrifugalized specimen is satisfactory. The cysts are somewhat concentrated by the centrifugalization. Placing a cover glass on the preparation and thus thinning it out is a little more satisfactory, especially for one without much experience. The cysts can be seen with the low power lens as round or oval, glistening white, hyaline objects, somewhat larger than pus cells.

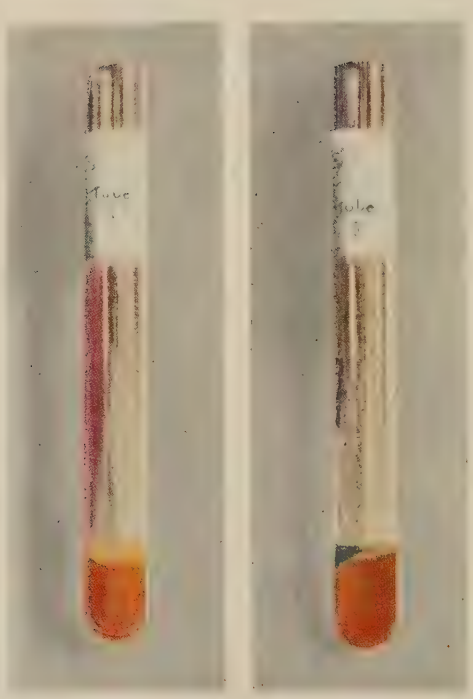
For definite identification and differentiation from cysts of Endameba coli, they should be stained with iodine and examined under the high dry lens or the oil immersion. The centrifugalized specimen is best for this purpose. To the sediment in a tube from which all except a few drops of water has been poured off, add a few drops of Lugol's solution. Transfer a small portion of this stained sediment to a slide, put on a cover-glass, pressing this firmly to thin out well, and examine with the high dry or the oil immersion lens.

The most important differential point is the number of nuclei which can now be recognized much better than they can be in unstained material. Cysts of Endameba coli when fully matured contain 1 to 8 nuclei, those of histolytica 1 to 4, those of Endolimax nana 1 to 4. and those of Iodameba williamsi only 1.

While the cysts of Endolimax nana contain the same number of nuclei as the pathogenic variety, they are very small and the nuclei can only be made out with considerable difficulty when viewed with the oil immersion lens.

Interpretation. In active or acute amebiasis pathogenic amebas can always be found upon proper examination. Thereby it is possible to determine that an acute case of dysentery is or is not due to ameba. During a quiescent stage of the disease, when few ulcers remain and when they are almost healed, it may be impossible to find vegetative amebas, because of the very small number present, and the absence of the kind of material in which they may be found. Cysts (1 to 4 nucleated) practically always can be found in such quiescent cases, as well as in some others that have not had recognized

PLATE XV



Benzidin Test for Occult Blood
Tube I. Negative reaction.
Tube II. Positive reaction.

clinical symptoms. Presence of amebas or amebic cysts does not prove that symptoms complained of are due thereto, but absence of them is very strong evidence against existing symptoms being due to amebiasis. They should be found in practically all cases.

Occult blood

Test for occult blood. Hemorrhage may occur so high in the alimentary tract that the blood is so altered, or it may be present in such small quantity, that it cannot be recognized in the feces, either by macroscopic or microscopic examination. Such blood is called occult blood. Whenever practical, special precautions should be taken to avoid outside sources of blood that would give a positive reaction, such for instance as eating food containing blood, hemorrhages from the mouth and nose, or hemorrhoids, etc. A purgative followed by a meat-free diet a day or two before the specimen is collected insures against blood from food.

Technic of test. Make a suspenson of the materal in water, unless it is already sufficiently liquid. Ordinary formed feces would be diluted with about five to ten times as much water. Whenever specimens are contained in a proper bottle container, the best way to make the solution is to add some water in the bottle, replace the stopper, and shake until enough of the feces have been dissolved to make the proper consistency. It is not necessary that all of the feces should be dissolved. The suspension of feces prepared for centrifugalization in examining for ova is suitable for this purpose. The supernatant fluid following the first centrifugalization is especially suitable. Place in a test-tube a little benzidine (crystals). A dip on the point of a knife blade about half the size of a pea is ample. Add a few (5 or 6)drops of glacial acetic acid. Shake until benzidine is dissolved. Pour in about 0.5 to 1 cc. of the diluted feces. Shake to mix and then add a few drops (10 or 15) of fresh peroxide of hydrogen. It is better to allow this to trickle down the side of the tube and overlay the material being tested. If occult blood is present, a dark blue ring (Plate XIV) at the zone of contact forms within a minute or two. If none is present, no blue color appears. If the tube is shaken, the whole mixture turns blue, but the ring test is generally more striking.

Interpretation. A positive test shows the presence of blood in small or larger amounts. Occult blood is present in most cases of cancer of the stomach and cancer of the intestines all the time, and occasionally in cases of ulcer. Other possible sources of blood must be kept in mind in interpreting a positive test. A negative test showing the absence of occult blood is strong evidence against the existence of cancer, which is the chief value of the test. It is little evidence against ulcer, but whenever tests made on several different days are all negative, ulcer is usually not present. Feces of hookworm patients contain occult blood most of the time.

CHAPTER XI

Pus, Exudates and Similar Material

General remarks. It is often desirable to know what kind of bacteria, cells, etc., are present in various kinds of material, such as pus, exudates, etc. It is not considered within the scope of this book to go into bacteriology except to describe those simple methods of identification that can be carried out by those not equipped with a bacteriological laboratory, but who have the simple, comparatively inexpensive laboratory equipment that every practicing physician should have.

There are certain simple tests that may be made which enable one to determine in many cases to what group of organisms bacteria that are found belong and to identify them sufficiently accurately for practical purposes. One of the distinguishing features of germs is their shape (Fig. 99). We have bacilli which are rods of various shapes two or more times as long as they are thick, some of which are motile and others non-motile, and cocci which are round or oval, the length being less than twice the breadth. The cocci are further divided according to their arrangement with relation to each other into diplococci, arranged in pairs; staphylococci, arranged in bunches (grape bunch), and streptococci, arranged in chains. Still other classification according to arrangement, such as strepto-bacilli, strepto-diplococci, etc., is sometimes made. Classification according to morphology is based upon stained specimens.

Making the preparation and staining. When obtaining pus or exudates for examination with a view to discovering the causative germ, it is very important to obtain material from the seat of the disease process. For instance, it may be that there are many bacteria at the surface of a lesion which have nothing to do with the cause of the disease, but are simply growing secondarily in the pus and exudate. These bacteria may be much more numerous in the material at the surface, while in the diseased tissue itself none are to be found. Whenever it is desirable to examine a very fluid material for pus and bacteria, such, for instance, as urine, cerebro-spinal fluid, pleural or

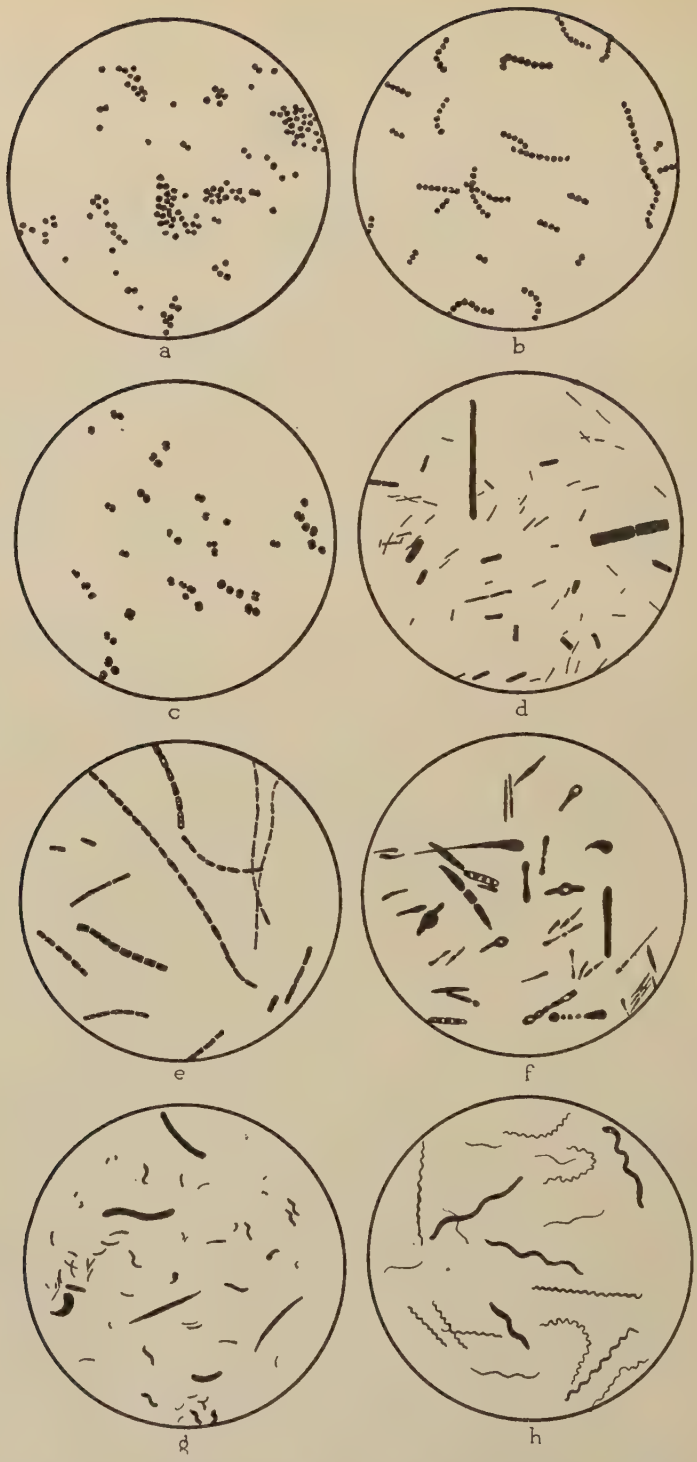


FIG. 99. Drawing illustrating morphological classification of bacteria. (a) Staphylococci; (b) streptococci; (c) diplococci; (d) bacilli; (e) streptobacilli; (f) diphtheroid bacilli; (g) spirillae, or curved bacilli; (h) spirochetae.

ascitic fluid, etc., it should be centrifugalized to concentrate the bacteria and cells. The supernatant fluid is drained off thoroughly (Fig. 100) and some of the sediment with which the preparation is to be made should be removed with a platinum loop.

For fresh unstained preparations a small amount of the material should be placed on a slide and covered with a cover-glass, which should be pressed down sufficiently to make the preparation thin

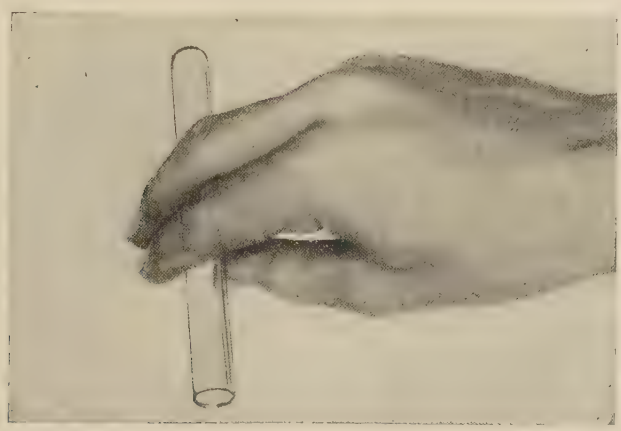


Fig. 100. Thoroughly draining sediment in bottom of centrifuge tube. Every drop of fluid is poured and shaken off.

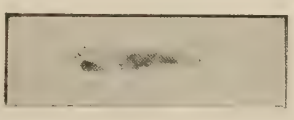


Fig. 101. Proper spread of pus or similar material to be stained and examined. There are thin and thick areas affording any thickness desired.

enough for examination. If the material is too thick it should be diluted with normal salt solution to proper consistency. Such unstained preparations must be examined with properly subdued light. Hyaline objects cannot be seen satisfactorily with strong light.

The beginner often mistakes Brownian motion for actual motility. In Brownian motion the objects appear to be dancing and moving actively, but except for currents in the fluid, they do not move from place to place. On the other hand, motile bacteria do move (swim

about) from place to place. It is good practice to examine a few old specimens of urine for bacteria.

In preparing specimens to be stained the material should be spread properly upon a slide (Fig. 101) and allowed to dry. A spread about one-fourth inch wide and about one or two inches long is very satisfactory. Such long narrow spreads lend themselves well to two or more different kinds or intensities of staining on the same slide.

FIG. 102. Touching the heated slide to the back of the hand to judge the temperature and avoid getting it hot enough to damage the film of material to be examined when fixing with heat.

Carbol-fuchsin and methylene blue stain. The best routine stain for preparations is the combined carbol-fuchsin and methylene blue stain, which we usually refer to as the "contrast stain." It shows up the cells and bacteria to the greatest advantage. The steps are:

1. Fix the dry film with heat.
2. Cover film with carbol-fuchsin for a few seconds. Wash.
3. Cover film with Löffler's methylene blue, one-fourth to one-half minute or longer. Wash, dry, and examine with oil immersion lens.

To fix, pass the slide, film side up, slowly through the flame of a

Bunsen burner or alcohol lamp two or three times. Do not heat any more than can be borne to touch the back of the hand without burning (Fig. 102).

The stain should cover the film only and not the entire slide. The best way is to touch the dropper of the drop bottle, with a drop of stain on it, to the film and spread (Fig. 103) the stain over the film as it is allowed to flow out of the bottle. Any desired preponderance of the red or blue stain can be obtained by varying the length of time the different stains are left on and by applying last the one desired to be most intense.

Gram's staining method. Most bacteria are either well stained by Gram's method (Gram-positive) or not stained at all by this method

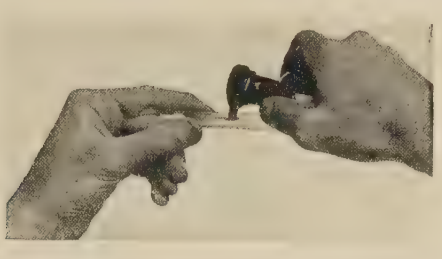


FIG. 103. Proper way to apply stain to a slide. Spread the stain over the material to be stained with the tip of the drop bottle stopper as it flows from the drop bottle.

(Gram-negative). Gram's stain does not show up the morphology of bacteria as well as the carbol-fuchsin and methylene blue stain does, and therefore is to be used largely for a further test or differentiation only after the other has been made and the kind of cells and the morphology of the bacteria has been observed.

The technic of Gram's stain as we do it follows:

1. Fix with alcohol for 1 minute.
2. Stain film with 0.5 per cent aqueous solution crystal-violet 1 or 2 minutes.
3. Pour off stain and cover film with one-fifth strength Lugol's solution about 1 minute.
4. Decolorize with acetone-ether mixture (acetone 70 per cent—ether 30 per cent). Wash.
5. Counterstain with one per cent safranin solution one-half minute. Wash, dry and examine with the oil immersion lens.

To decolorize hold the slide by one end. Let the other end be lower and drop (Fig. 104) decolorizer on the upper end of the film so that it runs over the film and off at the lower end of the slide. Only a dozen or two drops are necessary to decolorize.

If we should examine the preparation under the microscope after

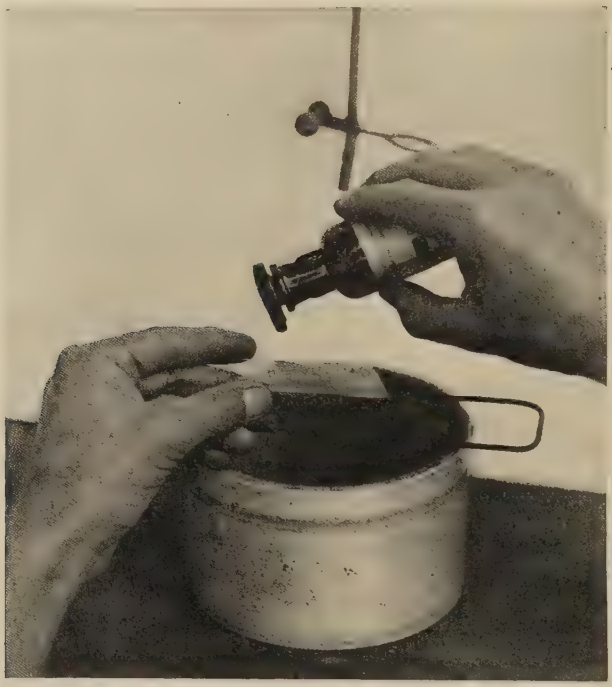


Fig. 104. Decolorizing with acetone-ether, acid or other decolorizing agent. The slide is held so that the fluid flows over the preparation and off at the end or corner of the slide.

decolorization we would find all Gram-positive bacteria stained a very deep violet, almost black, and the Gram-negative bacteria unstained. Unstained bacteria are difficult to see and we therefore counterstain with a stain that contrasts with the violet. The previously unstained objects take the counterstain and are now readily seen. There are several available counterstains, but we have found safranin as satisfactory as any. The carbol-fuchsin solution used in

staining tubercle bacilli makes a good counterstain when diluted about 1 to 10.

Acid-fast stain. Tubercle bacilli, lepra bacilli, smegma bacilli and certain other bacteria have a fatty capsule or covering. This is what gives them their most characteristic staining property known as "acid-fast." This fatty covering is not readily penetrated by ordinary stains. It is also resistant to alcohol and mineral acids. By employing a mordant with the stain and by the aid of heat, both of which increase the penetration by the stain, the bacilli can be stained within their fatty covering. The mordant usually employed is carbolic acid. When once stained, acid-fast bacteria are not decolorized by mineral acids like other bacteria that have no fatty covering, because the acid cannot penetrate the covering. This distinguishes tubercle bacilli and the other acid-fasts from most other bacilli and the different steps in the staining technic have this object in view.

The different steps are as follows:

1. Fix with heat.
2. Stain with carbol-fuchsin plus heat 1 minute.
3. Decolorize with 5% sulphuric acid solution. Wash.
4. Counterstain with Löffler's methylene blue. Wash, dry, and examine with oil immersion lens.

The fixation is accomplished by passing the slide slowly, film side up, through the flame of a Bunsen burner or alcohol lamp two or three times. Do not get the slide hotter than it can be borne on the back of the hand (Fig. 102). Use a very small flame. Hold the slide in the hand when fixing it.

Hold the slide by the edges near the end, between the thumb and the first finger of one hand, and put on the stain from a drop bottle held in the hand. Allow one drop of the stain to flow on the film and by means of the lip of the drop bottle spread (Fig. 103) it over the film, allowing more stain to flow out of the bottle as needed. A quantity equal to two, or at the most three, drops is sufficient for any slide. The stain should not be spread to the edge of the slide. It is extremely awkward to flood the slide with stain so that it runs off on the hands, table, etc.

After the stain has been placed on the slide it should be heated by passing it slowly through the flame two or three times. It must

not be boiled. Just let it get hot; this is sufficient. Forceps or special slide-holders are not necessary to hold the slide while heating (Fig. 105). Anybody who can't heat such a slide properly while holding it with his fingers without burning them or spilling the stain about, needs laboratory instruction or practice and not a slide holder. After heating, the slide may be placed on the slide-rest over the sink or waste jar for a minute while staining or it may be held in the hand.

Pour the stain off of the slide and drop on several drops of the acid solution. Allow the first few drops to run off (Fig. 104), and wash off the excess of stain that remained on the slide. Note that the red

FIG. 105. Heating in staining with carbol-fuchsin. Slide holders are unnecessary. Use a small flame and only enough stain to cover the area of the specimen to be stained.

color of the film fades and in less than a minute, becomes a very light pink or purple and ceases to fade more. It is now decolorized. By this decolorization the stain is taken out of all other bacteria, cells, etc., but if tubercle bacilli or other acid-fast bacteria are present they retain the stain. The slide is washed by running water over it.

This completes the staining process so far as the acid-fast bacteria are concerned, but the red-stained bacilli show up better against a contrast-stained background, and it often is desirable to have other bacteria and cells on the slide stained so they can be seen at the same time. Löffler's methylene blue is an excellent counterstain for this purpose. It should be put on and spread over the film in the same

way as the carbol-fuchsin. It usually stains sufficiently in less than one-half minute. Now wash well. Wipe the back, ends and sides of the slide dry. Take up any excess of water by "blotting" with the towel (or paper) and dry by warming it over the flame. Don't get it too hot.

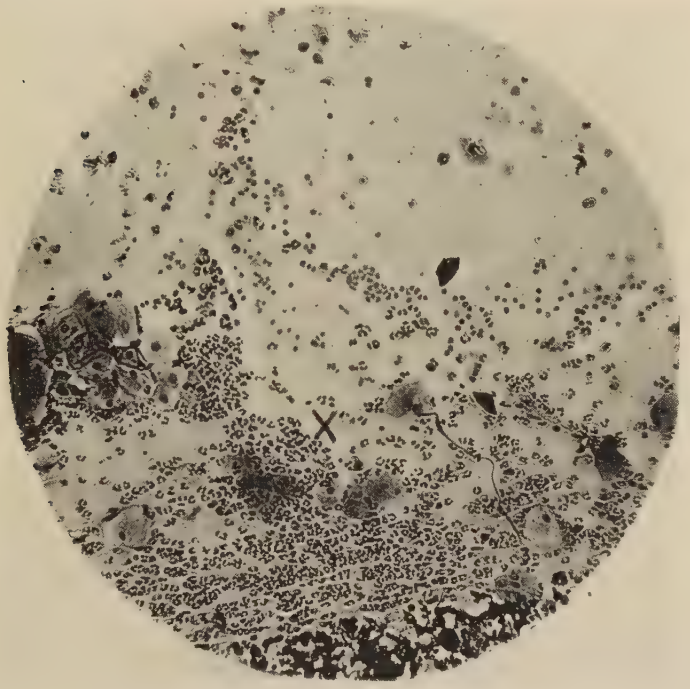


Fig. 106. Stained pus smear viewed under low power to locate proper area of evenly spread out pus cells for examination with oil immersion lens.

No cover-glass is required. Place some immersion oil on the slide and examine (Fig. 106) with the oil immersion lens for the red-stained bacilli.

Wright's stain should be used for staining scrapings from granulomatous lesions of cutaneous leishmaniasis, granuloma inguinale or chancroid for protozoan parasites, Ducrey's bacillus, etc. Allow the stain to act after dilution with water for 15 to 20 minutes.

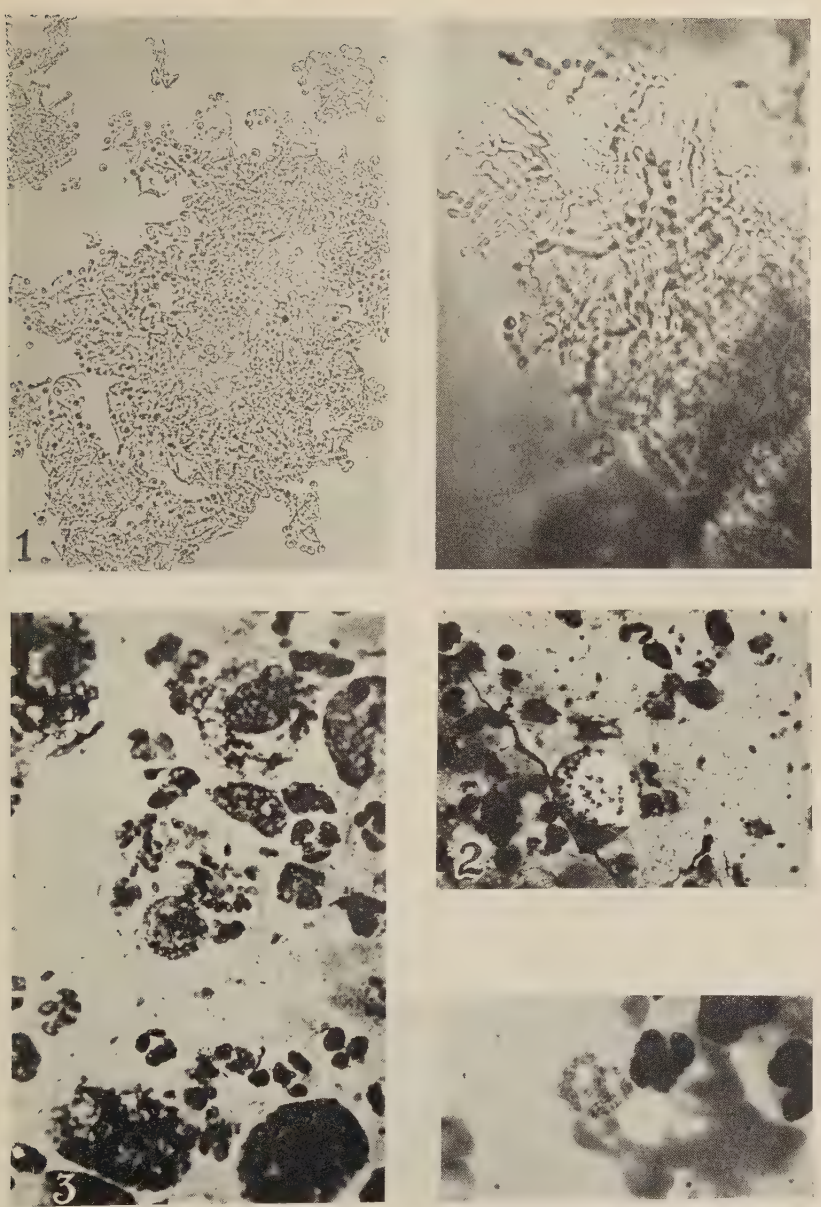
NAME	MORPHOLOGY	MOTILITY	STAINING REACTION	WHERE ORGANISMS MAY USUALLY BE DEMONSTRATED
1. Staphylococcus albus	Small, oval or round organisms; usually in clusters	None	Gram +	Pus from local infections. Normal skin
2. Staphylococcus aureus	Small, oval or round organisms; usually in clusters	None	Gram +	Pus from local infections. Normal skin
3. Staphylococcus citreus	Small, oval or round organisms; usually in clusters	None	Gram +	Pus from local infections. Normal skin
4. Streptococcus pyogenes	Small, oval or round organisms; usually found in chains	None	Gram +	Virulent local infections, showing spread via the lymphatics
5. Streptococcus mucosus	Large, oval organism, encapsulated; in chains	None	Gram +	Respiratory mucous membrane
6. Diplococcus pneumoniae (pneumococcus)	Slightly elongated cocci in pairs, surrounded by capsule. Often in chains	None	Gram +	Respiratory mucous membrane, and especially in sputum from lobar pneumonia
7. Diplococcus meningitidis (meningococcus)	Bean-shaped cocci, in pairs	None	Gram −	Spinal fluid in cerebro-spinal meningitis
8. Diplococcus gonorrhoeae (gonococcus)	Bean-shaped cocci, in pairs	None	Gram −	Pus from gonorrhoeal urethritis, vaginitis or ophthalmia
9. Micrococcus catarrhalis	Bean-shaped cocci, in pairs	None	Gram −	Respiratory or vaginal mucous membrane
10. Micrococcus tetragena	Bean-shaped cocci, usually occurring in fours. Large size	None	Gram +	Respiratory or vaginal mucous membrane
11. Bacillus anthracis (anthrax bacillus)	Large rods, single or in chains	None	Gram +	Blood of anthrax (charbon) patients
12. Bacillus subtilis	Large rods, single or in chains	Active	Gram +	Frequent contamination of blood, media, urine, etc.
13. Bacillus diphtheriae (Kleb's-Löffler-bacillus)	Beaded or granular rods, or clubs	None	Gram +	Suitable cultures from diphtheritic lesions in respiratory tract
14. Bacillus pseudo-diphtheriae	Beaded or granular rods, or clubs	None	Gram +	Respiratory mucous membrane
15. Bacillus coli (colon bacillus)	Short rods	Slight	Gram −	Urine, feces, sputum, etc.
16. Bacillus enteritidis	Short rods	Active	Gram −	Urine, feces, sputum, etc.
17. Bacillus typhosus (typhoid bacillus)	Short rods	Active	Gram −	Suitable cultures from blood, urine and feces of typhoid patients
18. Bacillus paratyphosus (paratyphoid A or B)	Short rods	Active	Gram −	Suitable cultures from blood, urine and feces of patients with paratyphoid fever
19. Bacillus dysenteriae	Short rods	None	Gram −	Suitable cultures from feces of patients with bacillary dysentery
20. Bacillus mucosus capsulatus	Short rods, capsule	None	Gram −	Respiratory mucous membrane

21. Bacillus pestis (plague bacillus)	Short, plump rods, with a clear center occasionally	None	Gram −	Enlarged lymph glands, or suitable cultures from blood in plague
22. Bacillus of "pink eye" (Koch-Weeks bacillus)	Small, thin rods	None	Gram −	Conjunctival pus in "pink-eye"
23. Bacillus tetani (tetanus bacillus)	Long, slender rods, with one end knobbed	None	Gram +	The primary lesion of tetanus (lockjaw)
24. Bacillus pertussis	Small, short oval rods	None	Gram −	In sputum from patients with pertussis (whooping-cough)
25. Boas-Oppler bacillus	Large, oval rods	None	Gram +	Stomach contents in reduction or absence of HCl
26. Bacillus pyocyaneus	Small, slender rods—varying in size—often chains	Active	Gram −	"Blue-pus" infection of wounds
27. Bacillus chanceroideus (bacillus of Ducrey)	Short, plump rods	None	Gram −	Pus from non-syphilitic ulcerative conditions of genitalia (chanceroides)
28. Bacillus tuberculosis (tubercle bacillus)	Slender, often slightly curved, rods	None	Acid fast	Sputum, or pus, from lesions of tuberculosis
29. Bacillus smegmae (smegma bacillus)	Slender, often slightly curved, rods	None	Acid fast	Material collected from under the male foreskin, or female genitalia
30. Bacillus [butter or grass]	Slender, often slightly curved, rods	None	Acid fast	Butter, greasy foods and fresh vegetables
31. Bacillus leprae (Hansen's bacillus)	Slender, often slightly curved, rods	None	Acid fast	Scraping from the nose, or nodules, or lesions of leprous patients
32. Spirillum cholerae (cholera vibrio)	Short, slightly twisted rods, or comma-shaped	Active	Gram −	Suitable cultures from feces of cholera patients

For demonstration of pathogenic fungi, scrapings or small pieces of tissue, finger nails, hairs, epithelial scales or crusts are digested in a 10 per cent solution of sodium hydroxide for several hours, mounted under a cover-glass and examined with the high dry lens.

Application in practice. By making the simple tests of morphology, motility and staining reaction, one often gains valuable information as to what class of bacteria are present and, what may actually be of more value, what bacteria are not present. On pages 134 and 135 there is a short list of common bacteria, with their staining reaction, morphology and motility.

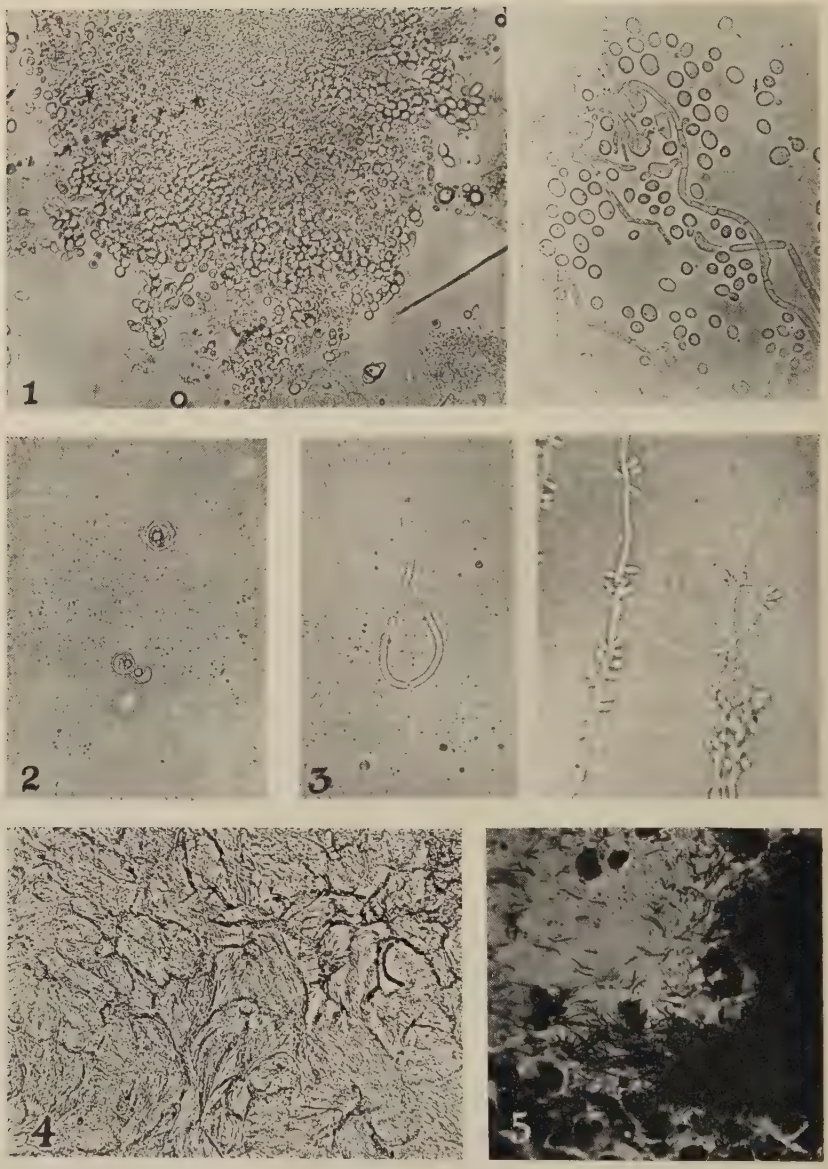
Plates XVI, XVII, XVIII, XIX, and XX, if studied with their legends, illustrate the appearance of some of the organisms, found in pus, exudates, scrapings, etc., from various diseases and conditions.



PHOTOMICROGRAPHS OF FUNGI AND LEISHMAN-DONOVAN BODIES

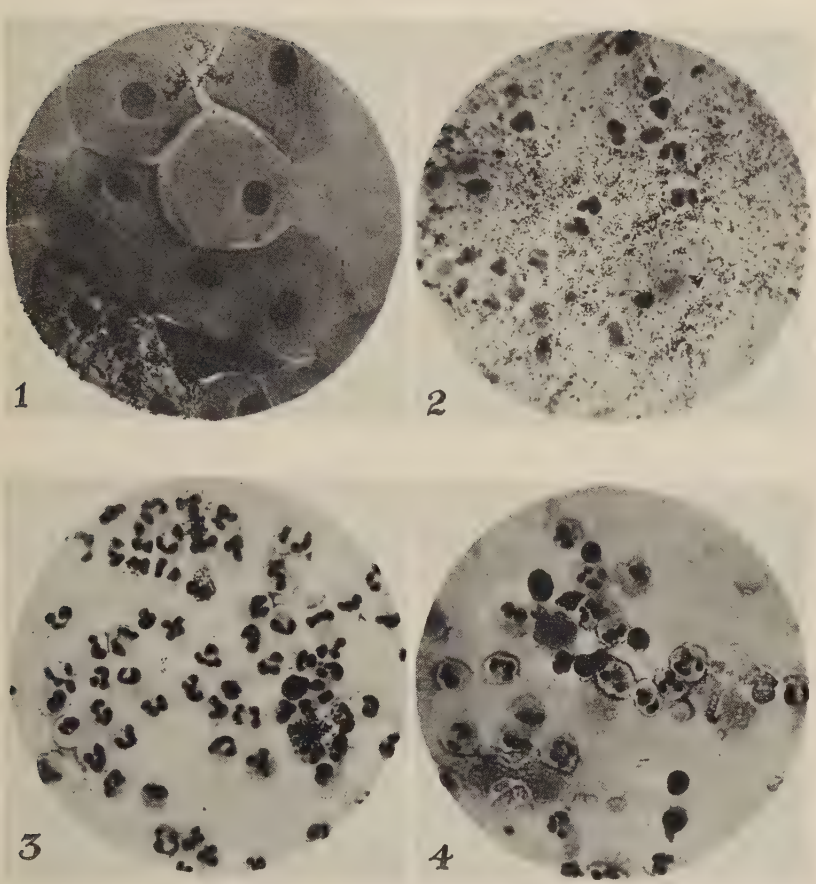
1. Scrapings suspended in caustic solution. Microsporons (Pityriasis versicolor) (left); Trichophytons (ring worm) (right).

2 AND 3. Scrapings, smeared—Wright's stain. 2. Leishmania tropica (Leishman-Donovan Bodies). 3. Donovan bodies in endothelial cells (left) and free (right).



PHOTOMICROGRAPHS OF PATHOGENIC FUNGI

1. Monilia (yeast) in sputum (left) and culture (right).
2. Blastomycetes.
3. Sporotricha. In pus (left) and culture (right).
4. Actinomycotic granule (high dry).
5. Actinomycetes. Stained (oil lens).



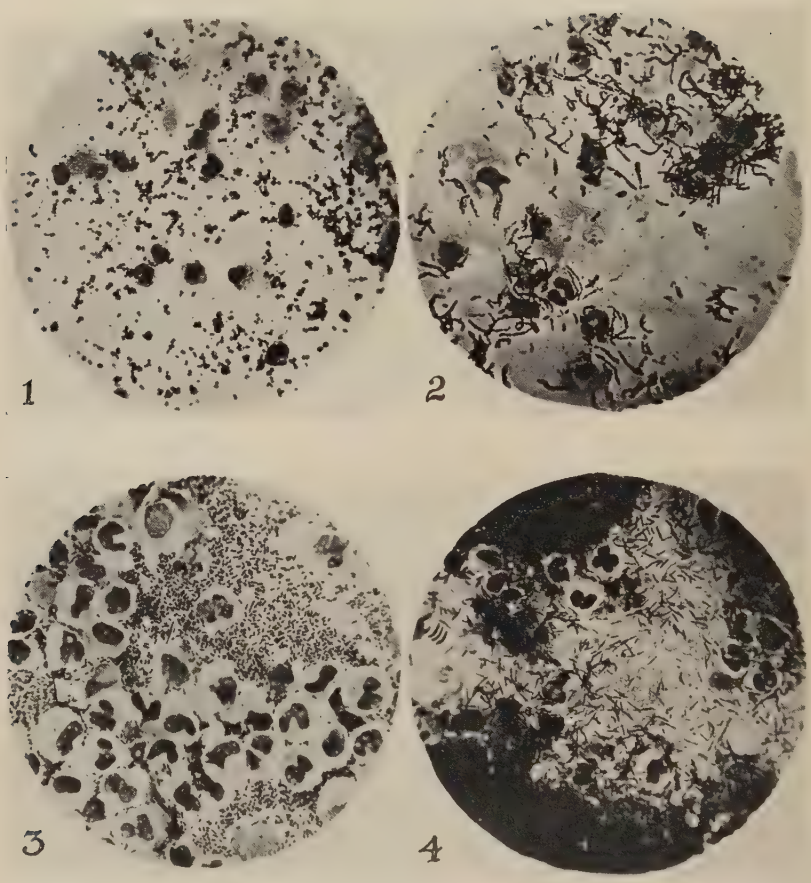
PHOTOMICROGRAPHS OF STAINED EXUDATES

1. Simple leucorrheal discharge. Epithelial cells and bacteria. Note absence of pus.

2. Chronic purulent leucorrhea. Note impossibility of specific bacteriological diagnosis from this *decomposing* material.

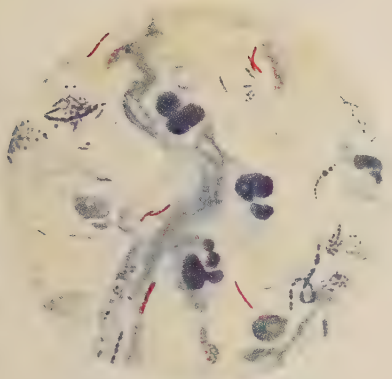
3. Gonococci in exudate from "within" the cervical canal of a chronic case.

4. Meningococci in sediment from centrifugalized spinal fluid. Note scarcity of cocci. Cell in center of field contains two pairs of cocci.

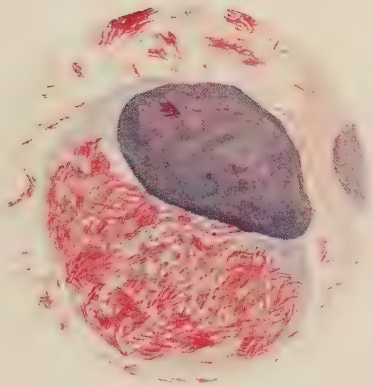


PHOTOMICROGRAPHS OF DIFFERENT TYPES OF BACTERIA IN EXUDATES ("CONTRAST" STAIN)

1. Staphylococci in prostatic secretion (centrifugalized).
2. Streptococci in sediment from catheterized urine.
3. Bacilli in sediment from catheterized urine.
4. Fusiform and diphtheroid bacilli. Spirochetes.



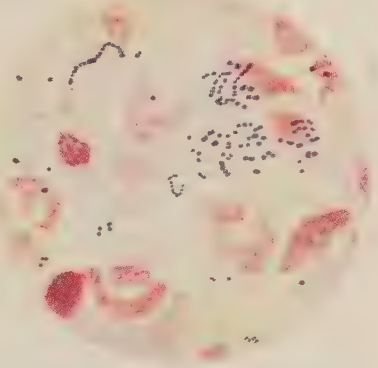
Tubercle bacilli



Lepra bacilli

Acid-fast stain

Gonococci



Pneumococci and staphylococci

Gram's stain

Colon bacilli

Fusiform bacilli and spirochetes

Contrast stain

DIFFERENT TYPES OF ORGANISMS IN PUS AND EXUDATES

CHAPTER XII

Sputum

Examination for tubercle bacilli. The practical laboratory diagnosis of pulmonary tuberculosis rests upon the demonstration of acid-fast bacilli in the sputum. There are several other acid-fast bacilli that resemble tubercle bacilli morphologically and otherwise, and therefore the laboratory diagnosis is not absolute. The identity of the bacilli could be determined absolutely by sufficient laboratory investigation, but the time and facilities required prevent it from being practical for ordinary purposes.

Collecting specimens. Many a failure to find tubercle bacilli in cases of tuberculosis or causative organisms in other diseases has resulted from the examination of improper specimens, such as saliva or secretion from the posterior nares containing no sputum from the lungs whatever. At the time specimens are requested for examination the patient should be informed that material that comes up from the lungs is wanted, and not saliva and nasal secretion. A mixture of several expectorations is desirable.

Most patients raise more sputum in the early morning, after a period of sleep and rest, than at any other time of day. Such sputum usually contains more tubercle bacilli, or other pathogenic organisms, than that raised at other times of day.

A clean, wide-mouth bottle, with new cork stopper, makes an appropriate container. Unless the container is furnished or specified, specimens are likely to be brought in the most inappropriate things, such as pasteboard boxes, small tin boxes, etc. Too small a quantity for the most thorough examination is likely to be brought, unless the patient is instructed.

Owing to chemical and bacteriological changes that take place in sputum upon standing, the characteristic staining reactions by which tubercle bacilli and other bacteria are recognized are frequently altered. In some specimens the bacilli begin to lose their staining characteristics within twenty-four to forty-eight hours. Specimens

of sputum, therefore, should not be more than twenty-four hours old —the fresher the better.

Whenever practical, it is better to have the patient cough up and expectorate sputum to be examined for tubercle bacilli, and for other examinations, while waiting in the office or laboratory. Generally, proper effort yields suitable specimens. A Petri dish or other suitable container should be provided for the patient to expectorate in.

Making the preparation. Tubercle bacilli are not present in all parts of a specimen of sputum. It is therefore necessary to make the preparation for examination from the kind of material most likely to contain bacilli. Many a failure to find tubercle bacilli in specimens

Fig. 107. Picking out favorable material from sputum in a Petri dish. Note use of two toothpicks.

containing them has resulted from examination of preparations made of material fished out of a bottle, jar, or other container with a platinum loop or other improper instrument. Pour out a proper quantity of the sputum into a Petri dish (we do not know of anything else as suitable). It is an advantage to set this on the table and place something (the cover of the dish) under one edge so as to tilt it and allow the sputum to flow to one side, leaving the remaining portion of the bottom not covered by sputum. This furnishes a convenient surface onto which particles may be drawn and teased out in order to separate the desirable from the undesirable material. Ordinary

flat-ended wood toothpicks are best for this purpose, as well as to spread the material upon the slide with. Use two picks (Fig. 107), one in each hand, and do not try to pick out material for examination with only one.

Select the gray, purulent particles to be seen in the sputum. With the picks drag the material onto the uncovered part of the bottom of the dish. Tease and separate from adhering mucus, saliva, froth, etc. Transfer to a slide. Get out several other particles from other parts of the sputum mass, mix, and spread all out on the slide. Thus is made a preparation representing favorable-looking material from several different parts of the sputum and much more likely to contain tubercle bacilli than one of similar size made from any one part of the sputum.

The spread of material on the slide should be comparatively large, but it should never reach the ends or edges of the slide. Burn the contaminated ends of picks and discard them.

The preparation must now be allowed to dry. There is no harm in warming the bottom side of the slide to hasten drying. As soon as the thinner areas on the slide are dry it is ready to stain. It is not necessary that the very thick masses sometimes present on such preparations should be perfectly dry before examining, as these are too thick to examine anyway.

For the technic of staining for tubercle bacilli, acid-fast stain, see page 131.

Description of tubercle bacilli. The bacilli (Plate XX) may be present in large numbers and in almost every field, or they may be so few that one or more are found only after several minutes' search. There often are clumps of several bacilli, but usually the number is small and many are found separate from others. It is common to see two or more bacilli lying parallel to or somewhat across each other. Two may be seen lying end to end. Most tubercle bacilli are slightly curved, and in many specimens they are slightly beaded in appearance.

Interpretation. It seldom occurs that any acid-fast bacilli are found in the sputum except tubercle bacilli. For all practical purposes acid-fast bacilli found in sputum, in the presence of clinical evidence of tuberculosis, are tubercle bacilli. A thorough examination of a proper specimen of sputum without finding any acid-fast

bacilli is evidence that the patient has not tuberculosis advanced to the stage in whch there is breaking down of lung tissue. It is not, however, proof of this, since it often occurs that bacilli are found only after repeated examinations. It is not possible, therefore, to determine with certainty that a patient has not tuberculosis by not finding tubercle bacilli, no matter how thorough the examination.

Examination for other bacteria and parasites in sputum. Selected material may be placed on a slide, covered with a cover glass, and examined, with subdued light with the low power or the high dry objective, for larvae of intestinal parasites, as strongyloides, uncinaria and ascaris, flagellates, lung flukeworm ova, amebas, actinomycetes, blastomycetes, yeasts (monilia) and other fungi, cells and debris, spirochetes, and bacteria. Some of the latter groups are better studied and recognized in stained specimens.

Staining. Care should be taken to select suitable material from the lungs. This should be picked and spread upon a slide in the same way as pus and similar material from other sources. The combined carbol-fuchsin and methylene blue stain recommended (page 128) for pus and exudates generally is most satisfactory. The cells and bacteria show up in a most satisfactory manner. Gram's stain (page 129) is often useful as an additional means of classifying bacteria found present. This method is useful in identifying pneumococci (Gram-positive) and influenza bacilli (Gram-negative), both pathogenic bacteria frequently found in sputum. By carefully selecting material that has been coughed up pneumococci can be demonstrated with much satisfaction in cases of pneumonia. While pneumococci are often found in material from the mouth, nose, and throat of persons who have no clinical symptoms, if they are found in large numbers in properly collected sputum in the presence of clinical evidence and physical signs, the conclusion is warranted that the disease is caused by pneumococci.

CHAPTER XIII

Leprosy

Technic of obtaining material and making preparations for examination. Lepra bacilli are present in the tissues of leprous lesions, and in the case of tubercular leprosy they are almost incredibly numerous. Since the bacilli are chiefly within fixed tissue cells, it is necessary to scrape up the tissue and examine this material for the bacilli. Most leprous lesions are anesthetic, and little or no pain is produced by

Fig. 108. Scraping leprous lesion. Bleeding is prevented by circumscribing the area by making pressure with the fingers.

the scraping. Catch up and squeeze (Fig. 108) the suspected tissue between the thumb and finger. With a scalpel scrape down into the tissue and secure scraped-up tissue, fairly free from blood. Blood is avoided by keeping the area anemic by the pressure made with the fingers. The edges of the lesion often contain the most bacilli. When tubercles are present, scrape them. Spread the scraped material on a slide and allow to dry.

Staining. Lepra bacilli are acid-fast and are to be stained exactly like tubercle bacilli (see page 131).

Appearance of lepra bacilli. Lepra bacilli (Plate XX) look like tubercle bacilli, to ordinary observation at least, but in most specimens the very much larger numbers are striking. Many of the bacilli are seen arranged in masses mostly in the so-called lepra cells. There is no staining or morphological difference between lepra bacilli and tubercle bacilli which permits positive differentiation of them.

The differentiation is based chiefly upon the source of the material and especially upon the enormous numbers of acid-fact bacilli characteristically packed into the cells.

Interpretation. Large numbers of acid-fast bacilli, especially if grouped like lepra bacilli, found in material scraped from tissues showing clinical evidence of leprosy, are for practical purposes lepra bacilli. The absence of acid-fast bacilli in scrapings from a tissue indicates very strongly, but does not prove infallibly, that the tissue is not leprous.

Lepra bacilli are almost always found in the nodular type and in ulcerative lesions. There may be few or no lepra bacilli found in material from the anesthetic erythematous lesions.

CHAPTER XIV

Spinal Fluid

Meningitis. The different forms of cerebrospinal meningitis can usually be easily diagnosed by proper microscopic examination of cerebrospinal fluid obtained by lumbar puncture. Since we now have a valuable specific remedy for at least one form (meningococcic), exact diagnosis becomes of great importance.

Obtaining material and making preparation. It is not considered within the scope of this book to describe the technic of lumbar puncture (Fig. 109). We would state, however, that it is a very simple operation, and easy to perform. The patient should be turned from side to side a few minutes before the puncture is made, in order to stir up the cells in the canal, which tend to settle to the lower side when the patient lies in one position for some time. The quantity of fluid drawn for diagnostic purposes should be 10 cc. It should be allowed to drop (Fig. 110) directly from the lumbar puncture needle into two clean test-tubes or small bottles. The first one is likely to contain some blood cells. Therefore, the second one should be used for examination, especially in making cell counts and globulin tests. The sooner the examination is made after the fluid is drawn the better, except in the case of tubercular meningitis, in which it is better to wait two or three hours for the pellicle of fibrin to form.

Sometimes the fluid is very cloudy and purulent, in which case smears should be made at once after it is drawn. When not quite cloudy it is necessary to centrifugalize the specimen, in order to concentrate the cells and bacteria. Centrifugalize long enough to thoroughly settle the suspended cells, etc., to the bottom. Pour off the supernatant clear fluid and drain well (Fig. 100). With platinum loop make proper spreads of the sediment on slides. A spread about one-quarter inch wide and one inch or more long is satisfactory. Allow this to dry in the air and then stain. Usually a pellicle of fibrin forms in fluid from tubercular meningitis upon standing for an hour or two. As this contracts it gathers tubercle bacilli in its meshes, if any are present. This should be "fished" out and spread

upon a slide or slides for examination. If no pellicle forms a centrifugalized specimen should be examined. A high speed centrifuge will throw down tubercle bacilli fairly well.

The best stain for all kinds of pus is the carbol-fuchsin and methylene blue stain (see page 128 for the technic). It is usually desirable to make a Gram's stain also (see page 129 for the technic). Whenever tubercle bacilli are to be looked for, the acid-fast stain is to be employed (see page 131 for the technic).

Fig. 109. Introducing needle in making lumbar puncture with patient in sitting position. The skin has been sterilized at the site of puncture with tincture of iodine, and after injecting a few drops of 1 per cent novocain into the skin.

Meningococci. Meningococci (Plate XVIII, Fig. 4) are intracellular (and extracellular) coffee-bean-shaped diplococci. In some specimens they are quite numerous, but in many others they are not. Frequently a search of many minutes is required to find a few pairs of cocci. It is a good idea not to diagnose as meningococci any organism that is not intracellular. The meningococcus is Gram-negative.

Pneumococci. In pneumococcal meningitis the pneumococci (Plate XX) are usually very numerous. They are chiefly extracellu-

lar, but many are intracellular. The lanceolate-shaped diplococcus, which is Gram-positive, is quite characteristic.

Tubercle bacilli. Tubercle bacilli from the cerebrospinal fluid (Plate XX) stain and look like the same organism from other sources. They are usually not numerous. The cellular increase is largely made up of mononuclear cells.

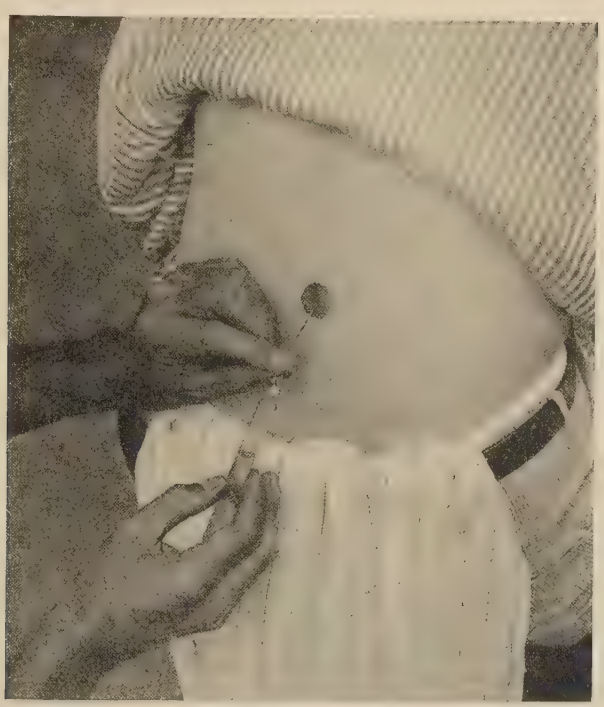


FIG. 110. The spinal fluid is allowed to drop directly into the centrifuge tube

Other bacteria. Other bacteria of various species may be, in rare instances, the cause of meningitis, and may be found in the cerebrospinal fluid. This may occur especially in traumatic meningitis.

Cell counts. To make a total cell count in spinal fluid make a preparation in the Bass-Johns counting chamber. Count all the cells in the entire ruled area. The number counted is the number per cubic millimeter. Usually no dilution is necessary in counting the cells in

those fluids in which cell counts are required—tuberculosis, poliomyelitis and syphilis, especially. If there are too many cells to count in an undiluted specimen, the fluid may be suitably diluted with Toison's fluid or salt solution, in which case the count must be multiplied by the dilution to get the total number per cubic millimeter in the undiluted fluid.

To make a differential cell count, drain off the supernatant fluid and make a spread on a slide of the cell sediment collected by centrifugalization. Stain either with Wright's stain or with the carbolfuchsin and methylene blue stain, and count as in counting blood.

Test for globulin increase. Put saturated aqueous solution of ammonium sulphate in a test-tube to a depth of about one inch. Overlay this carefully with spinal fluid which has been cleared of cells by centrifugalizing. A good way is to allow the fluid to flow down the side of the tube from a pipette. An increase of globulin is shown by a ring varying from a very faint whitish ring appearing not later than one hour to a very heavy ring appearing immediately.

Interpretation. The presence of meningitis and the causative organism can usually be diagnosed with satisfaction by proper microscopic examination. It sometimes happens that there are so few meningococci or tubercle bacilli present that these organisms may not be found. When no pus cells are present there is no meningitis produced by pyogenic organisms. When many pus cells are present and no bacteria found, the case is usually meningococcal meningitis.

Normal spinal fluid contains up to 10 cells per cubic millimeter, and these are all mononuclear cells. The count in poliomyelitis usually runs 100 to 300 cells with 5 to 10 per cent neutrophiles. In tubercular meningitis the count usually runs 300 to 1000 cells and approximately 100 per cent mononuclears. In tabes dorsalis there are usually 90 to 300 cells per cubic millimeter, and in paresis about 30 to 90, practically all of which are mononuclears. In meningitis due to any pyogenic organism the total count is enormously increased, nearly all the cells being neutrophiles.

Increase of globulin indicates some meningeal infection or disease, including poliomyelitis and syphilis.

CHAPTER XV

Diphtheria

Principles of laboratory diganosis of diphtheria. It is possible to make a practical diagnosis of diphtheria bacilli in the laboratory by reason of the fact that when grown upon certain culture media (Löffler's blood serum) they grow much more rapidly than the other

Fig. 111. Bass diphtheria culture tubes. The tube on the left has been broken open and the swab has been straightened out ready for use.

bacteria with which they are associated in the nose, mouth and throat, and that on this media they show characteristic morphology not shown by any other bacilli likely to be found in these places.

Material required. A tube of Löffler's blood serum and a sterile swab are required. Tubes and swabs are furnished by city and state board of health laboratories, but they soon deteriorate. A special hermetically sealed tube containing the swab also (Fig. 111) was devised by one of us (Bass) and is to be preferred, especially by those who have to buy their culture tubes. This tube is small, convenient,

and keeps indefinitely. One doing general or special practice of medicine who is likely to see cases of probable diphtheria should include one or more of these tubes in his armamentarium. If he carries a satchel on his visits to the homes of patients, it should contain one or two of these tubes. Any physician equipped with a microscope and not having access to a general bacteriological laboratory should keep such tubes on hand at all times. (These tubes may be purchased from I. L. Lyons & Co., New Orleans, La.)

Fig. 112. Removing cotton plug from culture tube preparatory to inoculating with the swab.

Making the culture. In cases where diphtheria is suspected there often is membrane or other local evidence of the disease, either on the tonsils, uvula, fauces or posterior nares. Often, however, no membrane is recognized, but still diphtheria is suspected and laboratory examination for diphtheria bacilli is desired. It is desirable to obtain material to be planted on the culture media from the surface

of the diseased mucous membrane, and as free as possible from bacteria and secretions from any other part. Most people who are old enough to make the effort can show the pharynx without the tongue depressor being used, by opening the mouth wide and making a gagging effort. At this moment the swab should be rubbed over the surface of the diseased part. In the case of a young child or a person who cannot expose the fauces properly, use of the tongue

Fig. 113. Inoculating culture media by rubbing swab over surface. Note how cotton plug is held.

depressor is necessary. In nasal diphtheria the swab should be passed into the nares and some of the secretion should be obtained. Now, holding the culture tube in the left hand and the swab in the right, remove the cotton plug from the tube (Fig. 112), rub the swab over the surface (Fig. 113) of the culture media gently and then replace the plug. The best way to dispose of the infected swab is to cast it into the fire if one is convenient, or it may be returned to the

empty end of the tube and all wrapped in paper to be destroyed at a more convenient time.

In spite of the duty the physician owes to his patient to be prepared for such work as this whenever it is indicated, it often happens that a culture tube is not at hand when the suspicious case is first seen. Instead of waiting until the next visit to get a culture tube, an extemporaneous swab may be made by winding a little absorbent cotton on a suitable stick and taking the culture with it. The inoculated swab is placed in a dry, clean, small, empty bottle or test-tube or in an envelope, and is carried to the office or laboratory, where a culture tube is inoculated in the way described above.

Incubation. The inoculated tube or culture, which is usually made at the bedside of the patient, should be started to incubating at once. No special laboratory facilities or incubator are required. The tube should be wrapped with paper to insure against the plug coming out, and placed in an inside (vest) pocket where the heat of the body keeps it warm enough to favor the growth of diphtheria bacilli. This is almost as good as an incubator and often much more convenient. The tube may be kept warm in the pajama pocket during the night. Incubation is necessary, because the bacilli grow very slowly and have not their characteristic morphology unless the culture is kept warm. This method of incubation is especially advantageous because it starts from the time the culture is first taken and it sometimes may permit the diagnosis to be made several hours earlier than if time is lost to get the specimen into an incubator.

Examination of culture. Whenever many bacilli are present they usually have grown to sufficient numbers in six or eight hours and have their characteristic morphology so that they may be recognized upon examination. No visible growth will be present at this time. If the exigencies of the case demand very early diagnosis the culture may be examined after six hours and very often the diagnosis can be made this early. We have occasionally been able to make the diagnosis in four hours. If no diphtheria bacilli can be found upon early examination, the incubation should be continued and subsequent examination made. An examination should be made after eighteen to twenty-four hours' incubation before a final negative diagnosis is made. Whenever very few bacilli are present in the material with which the culture is inoculated, it may be this long

before they have multiplied sufficiently to be found upon examination. After twenty-four hours other bacteria which may be present often overgrow the diphtheria bacilli and prevent satisfactory diagnosis. It is therefore desirable to make the final examination by the expiration of this period.

Making preparations for examination. A good platinum loop is very valuable in examining the culture. Often the loop of the wire is too large, not smooth, or otherwise improper. The loop can be

FIG. 114. Sterilizing the platinum loop in flame of Bunsen burner. Note proper size of loop and the micro-burner giving a small flame.

properly formed and shaped by bending the end of the platinum wire snugly one time around the end of a smoothly sharpened lead pencil just where the lead disappears under the wood. After the loop has been formed, bend it back sharply in order to bring the center of the loop in line with the straight wire. Once a good loop is provided, take care not to bend it out of shape. Working with a poor platinum loop puts one to considerable disadvantage in this kind of work; still, many do it.

The growth on the culture media is not fluid enough to spread well

on a slide and it is an advantage to dilute it (on the slide) with some water. Place near the middle of the slide approximately the smallest quantity of water that the platinum loop will hold. Now hold the culture in the left hand and the platinum loop handle in the right. Sterilize the loop (Fig. 114) by heating in the Bunsen-burner or alcohol flame. Remove the cotton plug (Fig. 115) from the tube and draw the loop over the surface (Fig. 116) of the culture, endeavor-

Fig. 115. Removing cotton plug preparatory to examining culture, sterile platinum loop held in hand ready for use.

ing to scrape off any growth present. Often little or no growth can be seen. Stir the material removed from the culture in the water on the slide and spread out in a thin long streak (Fig. 101). Too thick smears are not good. Now flame the loop again and replace the cotton plug. As soon as the preparation dries it is ready to be stained.

Staining. The steps in staining are:

1. Fix with heat.

2. Cover the entire spread with Löffler's methyleneblue and allow to stain approximately one-half to one minute. Wash, dry and examine with oil immersion lens.

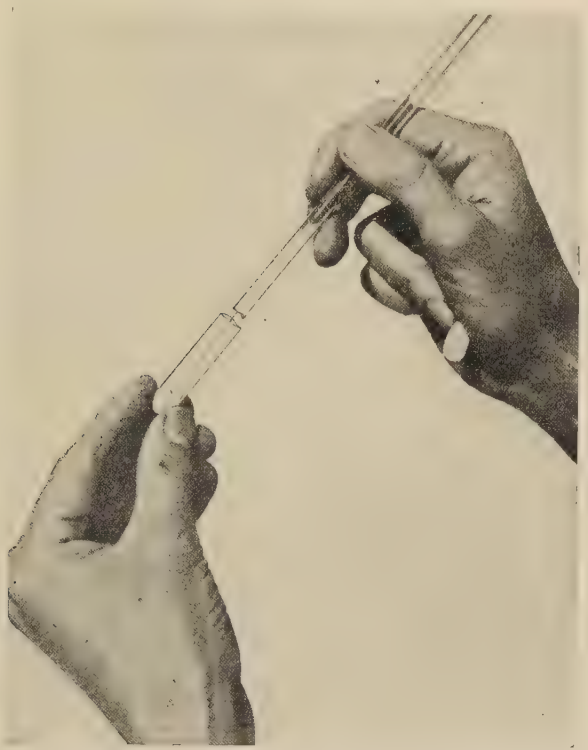


Fig. 116. Scraping the surface of the culture with the platinum loop to obtain material for examination.

To fix, pass through the flame slowly, film side up, two or three times. Don't get it hotter than can be borne on the back of the hand (Fig. 102).

It is good practice to stain suspected diphtheria preparations by Gram's method also. See page 129 for the technic. Diphtheria bacilli are Gram-positive.

Description of diphtheria bacilli

When taken directly from the seat of disease or when grown on ordinary media diphtheria bacilli cannot be differentiated by their morphology from many other species of bacilli likely to be found present in the mouth, nose and throat; but when grown on the

Fig. 117. Photomicrograph of diphtheria bacilli from throat culture

Löffler's blood serum media they present a morphology quite characteristic of this organism and a small group of so-called diphtheriod bacilli (Fig. 117). In the first place they are bacilli and no other type of organism (cocci, etc.) found need be considered as possibly diphtheria. They vary in their size from small up to very large. Some will be five or ten times longer or larger than others. Many

are club-shaped, others are more or less lanceolate-shaped on one or both ends. Most of the diphtheria bacilli are more or less beaded and in many this is so marked as to make the bacilli look somewhat like chains of streptococci. In addition to the beaded condition a variable proportion of the bacilli contain one, two, three or four much darker staining granules, called polar bodies. When only one or two are present in a bacillus they are usually situated near its ends. These polar bodies are much more numerous in some specimens than in others. They are fairly well shown with the methylene blue stain, but they are shown much better when stained by Gram's method. It is seldom that any other diphtheroid organisms show these polar bodies and this is a fairly reliable differential point, but is not infallible.

In most cases cultures that contain diphtheria bacilli contain other bacteria, cocci and possibly bacilli. Absolutely pure cultures are seldom obtained from diphtheria lesions.

Interpretation. Gram-positive diphtheroid bacilli found in the presence of clinical evidence of diphtheria are for all practical purposes diphtheria bacilli. A properly taken culture properly incubated and examined always shows diphtheria bacilli in the presence of the disease. In the absence of any clinical evidence whatever one should look with doubt upon diphtheroid bacilli. However, true diphtheria bacilli are sometimes found in persons (diphtheria carriers) who have no active symptoms, and in some having chronic purulent nasopharyngitis and otitis media.

CHAPTER XVI

Gonorrhea

Obtaining material and making preparation. During acute gonorrhea there is a great deal of pus formed, containing many gonococci. If the patient is a male the pus should be wiped from the meatus in order to get rid of the other bacteria that may be growing in the pus after it is exposed to external contamination. A fresh drop is brought to the meatus by "stripping" along the urethra. When there is very little pus being formed this is especially necessary. The fresh pus squeezed from the follicles in the urethra is much more desirable than old pus that has been standing under the prepuce or even in the urethra. In posterior urethritis it is necessary to "strip" from far back. In chronic gonorrhea where there is very little pus formed and most of that in the posterior urethra, seminal vesicles and prostate, this pus can usually be obtained by massaging and forcing it forward with the finger in the rectum.

A method of concentrating the cells and bacteria in prostatic material, described by one of us (Johns, in the *New Orleans Medical and Surgical Journal*, February 17, 1923, Vol. 80, pp. 463–464), is most useful. It consists essentially of centrifugalizing the material in a very small glass tube 2 to 4 minutes, and using the sediment thus collected. Those who are interested should consult the original article.

In acute gonorrhea in females the external pus should be wiped off from the orifice of the vagina and meatus, and fresh pus obtained from higher up. There are many other bacteria in the outer pus and gonococci often cannot be demonstrated in it with satisfaction. The best material for examination for gonococci in acute cases is obtained from higher up. The best material for examination for gonococci in such cases is obtained by introducing a finger into the vagina and "stripping" the urethra forward. In chronic cases pus may be obtained from the cervical canal or glands through a vaginal speculum. Ideal material can usually be obtained from the urethra or

cervical canal by scraping the walls with the edge of a blunt, grooved director or similar suitable instrument.

Sometimes it may be desirable to examine the urine for gonococci which are present in gonorrhoeal cystitis. Recently voided urine is *absolutely* necessary. (For technic, see pages 99 and 128.)

Staining. The best methods of staining for gonococci are the combined carbol-fuchsin and methylene blue stain in which they take the blue, while the pus cells are sharply brought out with combined red and blue, and the Gram's stain in which they take the red counterstain.

Appearance of gonococci. Gonococci are readily phagocytized by the polymorphonuclear leukocytes and therefore a large portion of those present in pus are within the pus cells (Plates XVIII and XX). There are no other bacteria for which gonococci are likely to be mistaken that are found intracellularly to the same extent. Although some gonococci are to be found outside of cells, it is best not to call anything a gonococcus that is not intracellular. The number in one cell varies from one to twenty or thirty or more pairs of cocci. The cells packed with diplococci found in acute gonorrhea are so typical that one who is at all familiar with them could hardly make a mistake. Many of the pus cells have no gonococci in them. In chronic gonorrhoea one may look over many fields of the microscope before he finds a pus cell with gonococci in it, while in acute gonorrhea practically every field has many.

Gonococci are always in pairs—true diplococci. The division between the two coffee-bean shaped organisms can be made out in most of them, but of course in some they are not seen in the proper position to show the division.

Interpretation. Intracellular, Gram-negative, diplococci from the urethra are for practical purposes gonococci, especially when typically arranged in the cells. In the absence of such diplococci an acute urethritis or an acute inflammation elsewhere is not gonorrhea. It is not possible to determine beyond doubt that gonorrhea (chronic) is not present in a given case, but thorough, repeated, negative examinations are strong evidence that it is not.

CHAPTER XVII

Syphilis

1. Examination for Treponema pallidum. It is of the utmost importance that a definite diagnosis, positive or negative, should be made in all cases in which syphilis is suspected, where lesions are present. It is most unjust and unfortunate for a person to be put on treatment on the basis of a guess diagnosis when a means of a certain diagnosis is so accessible. Diagnosis by inspection of early lesions of syphilis often remains more or less in doubt, but it can almost always be made with certainty by proper microscopic examination for Treponema pallidum. Those who assume the responsibility of the diagnosis and treatment of syphilis are under solemn obligation to their patients either to be prepared to make such examinations or to have them made by others who are prepared to do so.

The best and practically the only satisfactory method of examining for treponemas is with the dark-field microscope. Staining methods are unsatisfactory and frequently misleading.

Obtaining material and making preparations for examination with the dark-field microscope. The Treponema pallidum is present usually in very large numbers in all chancres, mucous patches, condylomata, adjacent swollen lymph nodes and in smaller numbers in other syphilitic skin lesions and most other syphilitic lesions of the body. It must be understood that the organisms are *in* the diseased tissue and not *on* it. In order to obtain material containing them, we must obtain it from the diseased tissue. In the case, for instance, of a chancre, it is necessary to obtain material from the hard syphilitic tissue and not simply from the indurated or ulcerated tissue over or around it. What is needed is "juice" from the diseased tissue and the scraped up tissue itself. It is very necessary to avoid getting a large amount of blood with the material.

To obtain proper material (Fig. 118) grasp the chancre or lesion between the thumb and forefinger and make sufficient pressure to drive out the blood, which is indicated by an anemic appearance of the tissue. Maintain the pressure and with a scalpel scrape into

the hard chancre tissue. Scrape up some of the tissue and squeeze out some "juice," which is usually rich in treponemas. Either transfer this to a slide with the scalpel or touch the slide to the drop of fluid that has been squeezed out. Do not let up on the pressure until the material has been secured for the reason that bleeding follows releasing the pressure and many blood cells are badly in the way.

Sometimes patients will not endure the pain produced. In case of an ulcerated lesion, a few crystals of cocaine may be applied to the ulcer to anesthetize it. Whenever lesions in the mouth are so situated on the lip or tongue that they can be manipulated in the

FIG. 118. Scraping chancre. Sufficient pressure is maintained to render the tissue anemic, prevent bleeding and squeeze out "juice" of the indurated base of the lesion which contains the largest numbers of treponemas.

same way, it should be done. Otherwise, it is better to pinch off a little of the diseased tissue with a suitable instrument, rinse it in salt solution and then crush it on a slide with the end of another slide, thus obtaining proper fluid from the diseased tissue. The diagnosis is less dependable whenever the lesion is in the mouth on account of the possibility of finding the non-pathogenic spirochetes commonly present in the mouth and mistaking them for Treponema pallidum. If the scraped-up material is too thick, it may be diluted with a little salt solution. It is preferable, however, to use only the "juice of the tissue" whenever sufficient can be secured and this can be done in nearly every case if proper technic is employed. Put on a cover-glass and press it down slightly. Do not use enough material to float the cover-glass. Use thin slides and cover-glasses.

Examination with the dark-field microscope. The principle (Fig. 119) of dark-ground illumination is that by means of a central stop in the condenser no direct light is permitted to pass through the object, while by means of a system of lenses and reflectors the object is illuminated by strong light reflected from the sides and at an angle. This is the same principle that makes dust particles visible in a beam of bright sunlight passing through a darkened room. In this way the particles suspended in the fluid are shown as very bright objects against a dark or black background.

The dark-field condenser fits in the substage carrier and takes the place of the Abbé condenser. It is necessary to have a dark-field condenser made for use with your particular make of microscope.

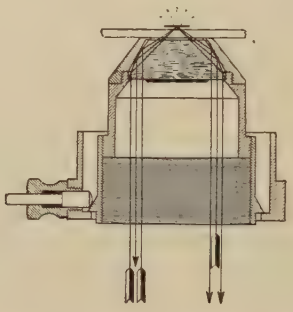


FIG. 119. Diagram indicating principle of dark-ground illumination and the path of rays through a dark-field condenser.

It may be ordered from the microscope manufacturer. In ordering be sure to give the number of your microscope. Printed instructions for use with the apparatus can be obtained from the manufacturer upon request and are very useful to the beginner.

The most recent type of dark-field condenser is illustrated in Fig. 121 and consists of a dark-field lens used in place of the top lens of the regular Abbé condenser supplied with the microscopes recommended in the front part of this book. Each of the manufacturers is prepared to supply this extra dark-field lens at any time for only slight additional cost. We are not prepared to recommend this type of darkfield for use in the diagnosis of syphilis.

A very strong light is necessary. Direct sunlight is entirely satisfactory when available. Those who do not have many of such examinations to make, and those who have not electric current, may

find this source of light sufficient to meet their needs. In most instances a time for the examination can be set when the sun is shining. The modern dark-field is supplied with a self-contained 12 c.p. electric light which operates through a rheostat and may be attached to any house lighting circuit (Fig. 120). The filament of this must be accurately centered with reference to the dark-field

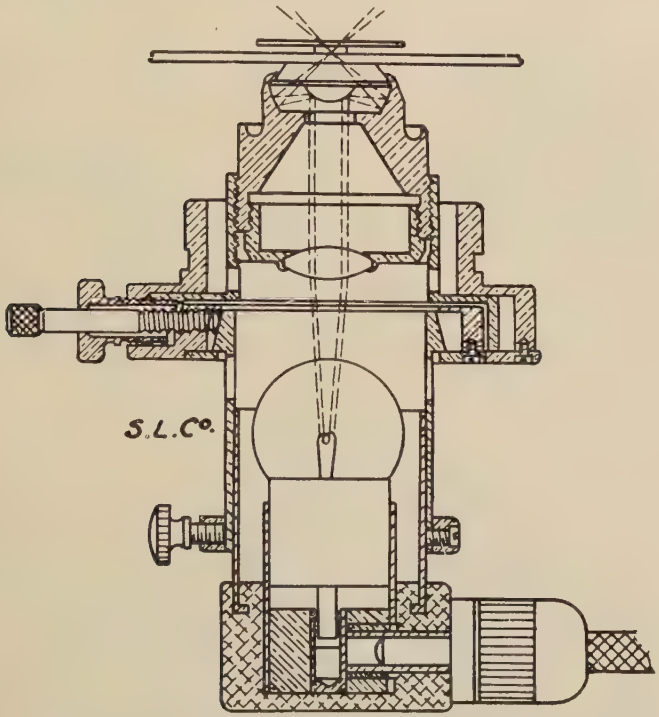


Fig. 120. Dark-field condenser, complete with self-contained light and rheostat.

condenser. When purchasing an outfit it is necessary to specify the type of current and voltage on which it is to be operated.

Remove the Abbé condenser and replace it with the dark-field condenser, taking care to see that the two thumb screws for centering are in front. Adjust the light so that a beam of light can be seen merging from the center of the condenser.

With the low dry lens focus upon the *surface* of the dark-field

condenser and find a small ring engraved in the center of the upper glass surface. By means of the centering screws, adjust it so that the ring is in the center of the field.

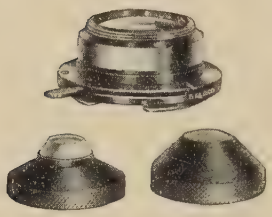


FIG. 121

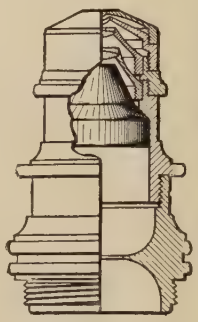


FIG. 122

FIG. 121. New type dark-field condenser to be used in place of top lens of Abbe-condenser. Bausch and Lomb. This cheaper instrument is not satisfactory for use in determining the presence or absence of treponemas in chancres.
FIG. 122. Funnel stop required in the oil immersion objective for dark-field work. Bausch and Lomb.

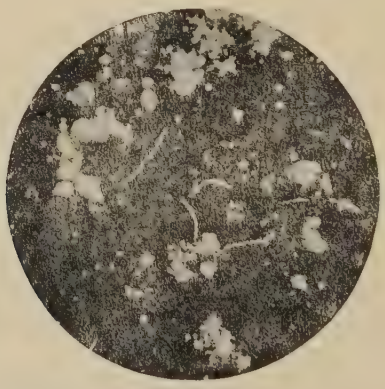


FIG. 123. Treponema pallidum in "chancre juice." Leitz dark-field.

Place a drop of water upon the condenser surface. Lower the condenser and place the slide on the stage, putting the part selected for examination in the center. Raise the condenser until the water is flattened out as the condenser is raised until it *almost* touches the slide.

Focus with the *low dry lens* and adjust the light by moving the mirror or raising or lowering the dark-field condenser until a brilliant spot of illumination is obtained in the exact center of the field. Unscrew the lens section from the hub of the oil immersion objective and put in the funnel stop (Fig. 122). Put a drop of cedar oil on the cover-glass and focus the oil lens. Re-adjust the light if necessary until brilliant dark-field illumination is obtained (Fig. 123), giving a brilliant white glow to all floating particles, against a black background.

There is a great deal of Brownian movement of the small particles suspended in the fluid under examination and we now see many things that cannot be seen by direct illumination. The suspended particles have more or less dancing motion. Spirochetes are seen in living, active state. They apparently revolve in cork-screw fashion and also move laterally. They have limited locomotion but are carried about more or less by currents. Experience soon enables one to recognize the organisms with certainty and satisfaction. One of the most satisfying laboratory examinations one can make is finding Treponema pallidum under the dark-field from a suspected syphilitic lesion.

One should learn to operate the apparatus while practising with material that may be obtained at any time and not wait until a chancre is at hand. Preparations made from material scraped from the gum margins between the teeth of almost any individual, which usually contains many spirochetes, are suitable for practice.

Differentiation between Treponema pallidum and other spirochetes. It is difficult to differentiate Treponema pallidum from some of the other spirochetes upon morphological differences. There are spirochetes in the mouth, nose, throat, rectum and vagina of a large per cent of all persons, but there are no non-pathogenic species on the skin. Spirochetes found in scrapings from lesions not on mucous membranes are therefore most certainly either pallidum or, in the very rare tropical disease, yaws, the Treponema pertenue. Spirochetes found in material from ulcerated lesions of mucous membranes are quite likely to be the common species found in the orifices of the body. If, however, the precaution is taken to thoroughly cleanse the surface before scraping, these are less likely to be encountered.

The Treponema pallidum has from about six to ten or more turns, while most of the other spirochetes have fewer. It is small and the

turns are short. There are five or more turns to the diameter of an erythrocyte. This is a convenient (although not absolute) guide, because in most any specimen we have a few erythrocytes to which any spirochetes found may be compared.

Gland puncture. The enlarged glands, inguinal and others, in early syphilis usually contain many treponemas, and gland puncture usually furnishes a little fluid which may show a good many. The puncture is a very simple operation. A good hypodermic syringe and 22 gauge needle are needed. The syringe and needle should be dry. Sterilize the skin over the intended site of puncture with tincture of iodine (an area one-fourth to one-half inch in diameter is large enough) and try to carry the point of the needle into the center of the gland. Make suction and rotate the needle a little. Stop the suction and withdraw the needle. Make preparations of the gland "juice" and examine in the same way as material obtained from other sources.

Interpretation. Small and actively motile spirochetes from a gland puncture or from a lesion not on a mucous membrane are for practical purposes Treponema pallidum and practically make a diagnosis of syphilis. A competent examination of a suspected lesion with the dark-field condenser for treponemas, if negative, is almost proof that it is not syphilis, but it is not infallible proof to this effect.

II. The Butler test—serum test for syphilis. There are a great many different modifications employed in different laboratories for making the Wassermann test. The exact original technic is seldom or never employed now. All methods are more or less complicated and require special apparatus and facilities and also considerable technical skill. For this reason and because of the many variable factors that enter into the test there is great variation in the accuracy and dependability of tests made in different laboratories of the country.

The Wassermann is a test for the presence or absence of a complement binding substance or property that is present in most cases of active untreated syphilis and nearly always absent when syphilis is absent. The Meinicke is a precipitin test which is positive in most cases of syphilis and nearly always negative when syphilis is absent. The two tests give practically identical results. The Butler test is a simple and very practical modification and improvement of the Meinicke precipitin test. It is a quick macroscopic slide test for syphilis corresponding to the Bass-Watkins macroscopic slide test

for typhoid. It runs parallel to the Wassermann test and for all practical purposes is as reliable for the diagnosis of syphilis as any of the different modifications of the Wassermann or precipitin tests. It has the advantage of reliability, extreme simplicity and comparative inexpensiveness in time and material. With the Butler test available, as it is, there is no excuse for not making serum tests

FIG. 124. Drawing blood from patient. First step is to distend veins by hanging the arm down and grasping it above the elbow. It is usually best to have the patient himself hold or constrict the arm.

for syphilis whenever needed. Those who do their own routine laboratory work can make this test about as easily and as reliably as they can any of the other laboratory examinations they make.

Material required. The antigen required is an alcoholic extract of veal heart to which a proper amount of cholesterin, benzoic acid and balsam of tolu has been added according to a formula recommended by Dr. H. W. Butler in the Journal of Laboratory Clinical

Medicine, Volume XIV, Number 4, pages 336 to 341, January, 1928. This antigen keeps indefinitely. At present there is no satisfactory commercial source of supply. Until such a source of

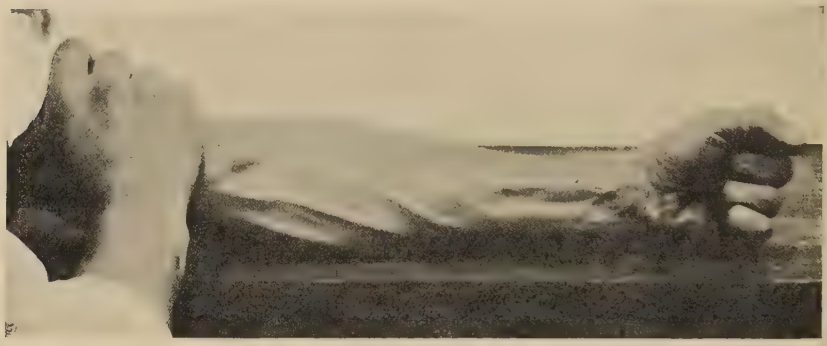


FIG. 125. Further distension of veins by patient clinching his fist. Iodine has been applied to the skin over the vein selected for puncture. Note the small area.

FIG. 126. Squeezing patient's arm to further distend the veins when necessary.

supply is established, it can be obtained from the Laboratory of Clinical Medicine, Tulane School of Medicine, New Orleans, La., at $3.00, cash with order, for 10 cc., which is sufficient to test about 200 bloods. The only other material required is a 3 per cent solution of

sodium chloride which you can make by dissolving 3 grams NaCl in 100 cc. of distilled water.

Apparatus required. The only apparatus required is such as is to be found in any office laboratory and consists of microscope slides, 1 cc. pipettes graduated in tenths, some medicine droppers, a small wide-mouth bottle of about 10 cc. capacity, and some wooden toothpicks.

Collecting blood from vein. Whenever as much as 1 cc. or more of blood is needed for this test or for other purposes, it should be drawn

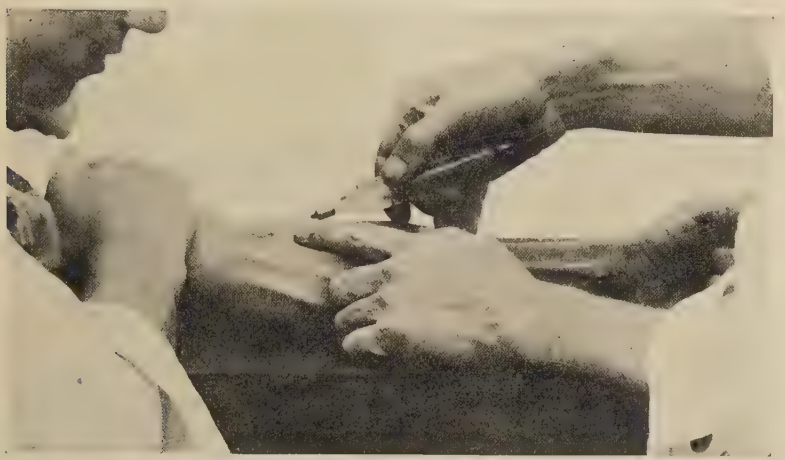


FIG. 127. Introducing the needle. Note proper angle of needle and manner of steadying the hand. Note also how the skin is pulled tight over the vein and in the opposite direction to the stick of the needle.

from a vein, usually at the bend of the elbow. Five cubic centimeters is sufficient for this purpose. The best apparatus to draw it with is a clean, *absolutely dry,* all-glass syringe and a 22 gauge stainless steel needle. It is not necessary that they should be sterilized. Sterilize the needle just before use by mopping it with tincture of iodine. Blood can be drawn with the needle alone by inserting it into the distended vein and allowing the blood to flow into a test-tube, but this is not so satisfactory. With the patient sitting, or if in bed, lying down, and the operator sitting in proper position, have the patient (or some one else) grasp the arm above the

elbow (Fig. 124). This is better than tying a constrictor around the arm. If held properly, this prevents the return flow of blood which now distends the veins. Have him clench the fist (Fig. 125) of the arm to be bled. This further distends the veins. If still not distended sufficiently, you can force more blood into them by gripping

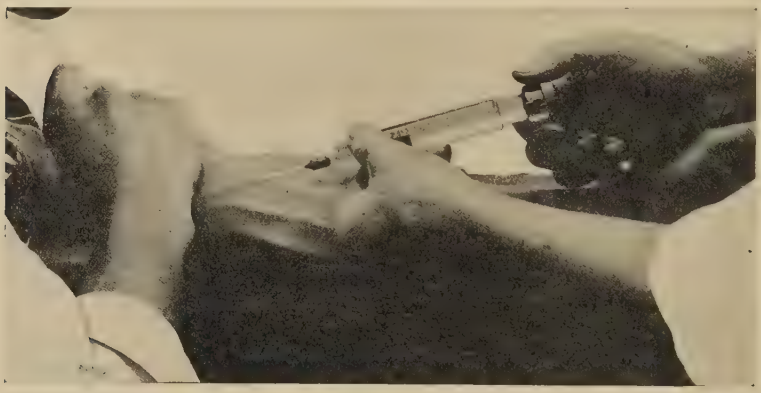


Fig. 128. Drawing the blood.

Fig. 129. Making pressure over site of puncture to prevent bleeding.

(Fig. 126) the arm just below the elbow. Now apply tincture of iodine to an area about the size of a silver dime over the point selected to stick the needle in. Do not apply it over a large area. With a finger of the left hand pull (Fig. 127) the skin taut over the vein and, holding the syringe in the right hand, which is steadied against the

arm of the patient, stick the needle at the proper angle into the vein. By deliberation and accuracy it is easy to strike the vein almost every time. Now draw (Fig. 128) the desired quantity of blood. Take care not to pull the needle out or stick it through the vein while drawing the blood.

Instruct patient to "let go" his grip. Then quickly withdraw the needle and make pressure (Fig. 129) over the puncture for a minute or two with a corner of a towel or piece of gauze. This prevents subcutaneous hemorrhage that otherwise often occurs.

Now remove the needle from the syringe and force the blood into a clean, dry test-tube before it has time to clot in the syringe. Do not use a syringe that is also used for giving medicines by injection.

Remove the iodine stain from the arm of the patient with a few drops of alcohol. Clean up the syringe before the blood clots in the needle.

The blood may be tested with the Butler test any time after it is collected. It is not necessary to keep it on ice if it will be tested within twenty-four hours.

Such specimens may be sent to other laboratories by mail for examination when you are not prepared to make the test yourself. Stopper the tube with a new cork, pack carefully in a mailing case, seal and send by special delivery, first class mail. Do not register or send by parcel post. Do not send to arrive on a Sunday or a holiday for fear of delay in delivery.

Method of making the Butler test. Always have slides, tubes, pipettes, etc., thoroughly clean before they are used. Any foreign material introduced might affect the accuracy of the test. It is better to have a separate pipette for measuring antigen and salt solution and a separate dropper for the diluted antigen, which are not used for any other purpose.

1. Measure 0.3 cc. of 3 per cent salt solution into the 10 cc. bottle for antigen dilution. Add 0.1 cc. antigen and shake the bottle at once. This makes a milky emulsion which is the diluted antigen used in the test. It is good for use during the next few hours, but should not be used the next day. This amount is sufficient for only two or three tests. If a larger number of bloods are to be tested, a correspondingly larger amount of antigen dilution should be made up.

2. Usually the serum will have already separated from the clot so that the serum can be taken up with a dropper. If not, the tube containing the blood should be centrifugalized. Place 3 drops of *crystal clear* serum on a microscope slide near the middle and place 3 drops of the diluted antigen near but not touching the serum. Promptly mix the two together by stirring with a wooden toothpick. Put the slide

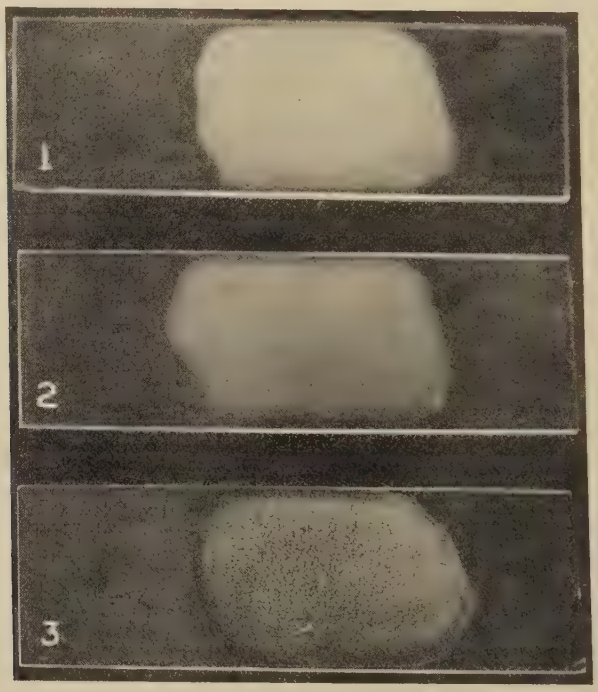


FIG. 130. Butler precipitin test for syphilis. 1, negative; 2, weakly positive; 3, positive.

down on the table for the reaction to take place. This occurs more quickly in some specimens than others. Most strongly positives show up and can be read within 3 to 5 minutes and often in even less time. Weaker bloods and some that are finally strongly positive require 15 or 20 minutes. This seems to depend somewhat upon the rate of evaporation on the slide. It is desirable to allow the material

to partially dry up on the slide before making a final negative reading, but it must not be allowed to dry so much but that at least half of it will flow when the slide is tilted.

3. To read the test, take up the slide and tilt it from side to side and from end to end sufficiently to cause the fluid to flow back and forth. This agitation increases the size of the granules and the visibility of the granular sediment or precipitate which forms when the test is positive (Fig. 130). No such sediment forms when the test is negative. The sediment is best seen by holding the slide so as to have a dark background in the distance and light from a window or, better, from a lamp falling on it from above and to the side. A little practice should teach one how to look at the slide so the sediment can be seen best. The reaction may be strong, weak or negative. The stronger the reaction the coarser the granules and the heavier the sediment. There are instances in which the reaction is doubtful just as is the case with all other serum tests.

Interpretation. The Butler test has the same significance and should be interpreted in the same way as the Wassermann test. Practically all cases of active syphilis after the appearance of the secondaries give positive reactions. Practically all non-syphilitic persons give negative reactions. As the disease improves under treatment, or as it becomes chronic, there is less and less chance of the blood giving a positive reaction and the reaction grows weaker. The strength of the reaction corresponds generally to the extent and activity of the disease Many cases of tertiary syphilis and uncured syphilis give negative reactions On account of the fact that there are some syphilitics who give negative reactions and an occasional non-syphilitic who gives a positive reaction, the test cannot be relied upon for infallible diagnosis of the disease or for an infallible contrary diagnosis. It must be considered in connection with the clinical evidence in the case for it to be most valuable. When thus considered it is often of much value.

The test is usually positive after the third or fourth week following the appearance of the chancre and always after the appearance of the secondaries. It should not be allowed to take the place of the much more reliable examination for Treponema pallidum when chancres or other active lesions are present.

APPENDIX I

Formulas

1. Wright's staining solution. Purchase 10 grams Wright's stain powder made by National Aniline and Chemical Company and a 500 cc. bottle of Mallinckrodt's absolute methyl alcohol. Put 0.9 gram of the powdered stain in the 500 cc. of alcohol, put in a few glass beads, stopper the bottle and shake thoroughly. Shake up the bottle every day or two for the next few days. This makes a large supply of Wright's staining solution (Wright's stain) at very much less cost than the "ready for use" stain that is often of very poor quality, as well.

2. Toison's fluid:

Sodium chloride	1 gram
Sodium sulphate	8 grams
Glycerine, C.P.	30 cc.
Methyl violet 5B (This must be Grubler's)	0.025 gram
Water	150 cc.

Mix. Keep this in stock bottle from which pour off (without shaking) small quantities as needed for use.

3. Standard dextrose solution for blood sugar test. Dissolve 0.1 gram pure dry dextrose in 500 cc. saturated solution of picric acid. Dextrose and picric acid for this purpose, of highest purity, are supplied by Special Chemicals Company, Highland Park, Illinois.

4. Fehling's solution:

Alkaline solution (A):

Sodium-potassium tartrate	173 grams
Sodium hydroxide	125 grams
Water	500 cc.

Mix and filter.

Copper solution (B):

Pure crystals cupric sulphate	36.4 grams
Water	500 cc.

Mix and filter.

The above solutions should be kept in separate bottles and equal quantities of each should be mixed when needed for use. This mixture constitutes Fehling's test solution.

5. Benedict's qualitative solution:

Copper sulphate (pure, crystallized)	8.7 grams
Sodium (or potassium) citrate	86.5 grams
Sodium carbonate (crystallized)	100 grams
Distilled water to make	500 cc.

The citrate and carbonate are dissolved together (with the aid of heat) in about 350 cc. of water. The copper sulphate is dissolved separately in about 50 cc. of water and then poured slowly into the first solution, with constant stirring. The mixture is then cooled and diluted to 500 cc. The reagent keeps indefinitely. It cannot be used for quantitative estimations.

6. Benedict's quantitative solution:

Copper sulphate (pure crystallized)	9 grams
Sodium carbonate (crystallized)	100 grams
Sodium (or potassium) citrate	100 grams
Potassium sulphocyanate	62.5 grams
Potassium ferrocyanid, 5 per cent solution	2.5 cc.
Distilled water to make	500 cc.

The carbonate, citrate and sulphocyanate are dissolved together (with the aid of heat) in about 400 cc. of water. The copper sulphate is dissolved separately in about 50 cc. of water and then poured slowly into the first solution, with constant stirring. The ferrocyanid solution is added, after which the mixture is cooled and diluted to 500 cc. One cc. of this reagent is reduced by 0.002 gm. of glucose.

7. Carbol-fuchsin staining solution:

Basic fuchsin	1 gram
Alcohol (95 per cent)	10 cc.
Carbolic acid, 5 per cent solution	100 cc.

Mix.

8. Loffler's methylene blue solution:

Methylene blue	1 gram
Alcohol (95 per cent)	60 cc.
Potassium hydroxide solution 1–10,000	200 cc.

Mix.

NOTE.—Aniline dyes made by the National Aniline and Chemical Company in America have been found satisfactory.

APPENDIX II

List of Apparatus Recommended

1. Microscope. Figs. 1, 2, 3, 4, 5, 6.
2. Dark-field condenser, with self-contained light and rheostat, if diagnosis of suspected syphilitic lesions is to be made. Fig. 120.
3. Electric light, 25 Watt, round frosted globe and some holder or other arrangement which permits bringing light as near as needed to the microscope. Fig. 9.
4. Blood sticker, stainless steel blade. Fig. 16. I. L. Lyons and Co., or The Surgical Supply Company, New Orleans, La.
5. 1 (or 2) box of 50 microscope slides, medium thickness, good quality.
6. 1 box cover-glasses, $\frac{7}{8}$ inch square, No. 2.
7. Water-bottle, tubing and Mohr's pinchcock arranged as shown in Fig. 131. In ordering, specify "water-bottle, tubing and pinchcock described in Clinical Laboratory Diagnosis, Bass and Johns, Fig. 131."
8. Diluting pipette for counting blood, 1 to 100, sometimes called "red cell" pipette. Fig. 37.
9. Bass-Johns counting chamber. Made by Bausch & Lomb Optical Company.
10. Dare hemoglobinometer, with self-contained light (Rieker Instrument Co., Philadelphia), Fig. 59, or Tallquist hemoglobin scale, Plate IV.
11. 3 Medicine droppers, long, for use in making typhoid agglutination test, Butler test, etc.
12. Box ordinary toothpicks, best quality, flat end.
13. 3 dozen test tubes, lipless, Pyrex glass, 12 mm. x 115 mm. No other tubes are required for any of the tests described in this book. Do not accept any other kind or size.
14. 1 micro Bunsen burner with 2 feet of pure gum tubing. Fig. 132.
15. 1 Squibb's urinometer. Fig. 71.
16. 1 tube litmus paper strips, red.
17. 1 tube litmus paper strips, blue.

18. Electric centrifuge. Those made by Internationa Equipment Co., Cambridge, Mass., have proven very satisfactory. Fig. 81. A hand centrifuge may be used, but it is a poor substitute. Fig. 83.

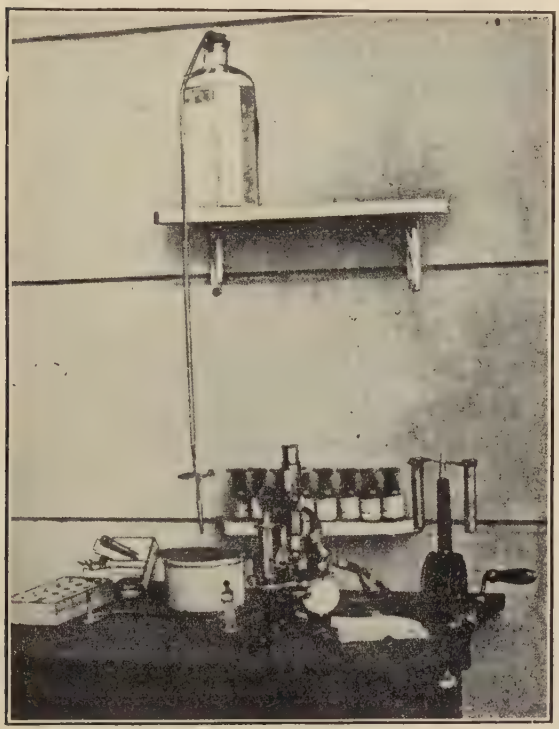


Fig. 131. Arrangement of work table where electric light is used, showing most of the material and apparatus required in ordinary microscopic work. It is preferable to have the centrifuge located on another table or shelf and not as here shown. Note arrangement of water bottle, waste jar and slide rest across it. Note that the tip of the tube from the water bottle is about one inch above the slide rest. Have yours so arranged. The tube may be all rubber except the bent glass tube that goes to the bottom of the bottle and the tip, instead of the intermediate glass tubing as here shown.

19. 1 glass funnel, $2\frac{1}{2}$ inch.
20. 3 Petri dishes, with covers, about 10 cm.
21. 1 platinum loop (platino-iridium), 26 gauge wire. Fig. 114.

22. 1 all-glass syringe, 10 cc., with stainless steel needle, 22 gauge.
23. 1 all-glass syringe, 2 cc., with stainless steel needle, 24 gauge.
24. 1 dozen T. K. drop bottles with flat top. Fig. 133. (Accept no other.) Fig. 134. 30 cc. size.
25. ½ dozen T. K. drop bottles, with flat top, 60 cc.

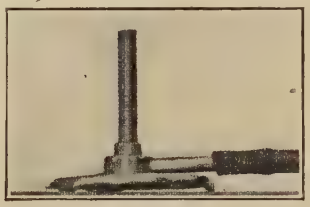


Fig. 132. Micro burner, much better than the larger Bunsen burners.

Fig. 133. Proper drop bottle for stains, reagents, etc.

26. Duboscq type colorimeter, if blood chemistry is to be done. Fig. 61. Bausch and Lomb Optical Co., Rochester, N. Y. This is also useful in making phenolsulphonephthalein renal function test. For the latter test, Dunning's colorimeter (comparometer) is satisfactory and less expensive. Hynson, Westcott and Dunning, Baltimore, Md.
27. 2 Purdy centrifuge tubes, graduated.

28. 5 graduated pipettes, 1 cc., graduated in 1/100.
29. 1 graduated pipette, 10 cc. graduated in 1/10.
30. 1 graduated cylinder, 1000 cc.
31. 1 graduated cylinder, 500 cc.
32. 1 graduated cylinder, 100 cc.
33. 2 beakers, 250 cc.

Fig. 134. Two kinds of improper drop bottles.

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